Roots and Drought and Breeding Better Crops

Adam Price
The vision

A better world without poverty…
  of diet
  of environment
  of aspiration
  of opportunity
  of culture

Through better roots????

Designer crops targeted to every farmer based on;
  climate and soil properties
  crop agronomy
  crop genetics
  marker assisted breeding
  GM technology
Contents

Work by our group on rice

Prospects

Challenges and solution
An F₆ mapping population

- Drought Screening
- Root Growth Screening
- Marker Assisted Selection
- Genes Behind Root QTLs
  - QTL accuracy
  - Genome Sequence and Annotation
  - Transcriptomics
  - Sequencing

- Meta-analysis
- MAS Breeding
- Near Isogenic Lines

Roots & Drought?
An F₆ mapping population

Drought Screening

Root Growth Screening

Marker Assisted Selection

Genes Behind Root QTLs

Meta-analysis

MAS Breeding

Near Isogenic Lines

QTL accuracy

Genome Sequence and Annotation

Transcriptomics

Sequencing
An F₆ mapping population

Drought Screening
Root Growth Screening
Marker Assisted Selection
Genes Behind Root QTLs

Meta-analysis
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Near Isogenic Lines
QTL accuracy
Genome Sequence and Annotation
Transcriptomics
Sequencing
The population of Recombinant Inbred Lines
Bala x Azucena

205 F₆ lines

Bala x Azucena

F₁ (self) 1 Individual

F₂ F₂ F₂ F₂ F₂ (self) 205 individuals

F₃ F₃ F₃ F₃ F₃ (self) 205 individuals

F₄ F₄ F₄ F₄ F₄ (self) 205 individuals

F₅ F₅ F₅ F₅ F₅ (self) 205 individuals

F₆ F₆ F₆ F₆ F₆ 205 families
An F₆ mapping population

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Hydroponics (unpublished)

Root penetration
Price et al. 2000, TAG
2 screens

Soil box
Price et al. 1999

Agar- gravitropism
(Submitted)

80% wax layer
Sand
Rhizotrons (Price et al. 2002)

2 treatments
2 years
= 4 screens
Soil box
(MacMillan et al. 2006)
4 treatments
8 screens
Field (WARDA)
(Cairns PhD thesis)
2 sites
5 screens
An F6 mapping population

Drought Screening

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- Root Growth Screening
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Drought Screening at IRRI and WARDA

• Tested at IRRI 1996 and 1998

• Tested at WARDA 1997 and 1998

• Evaluated for visible symptoms of drought stress and RWC

Price et al. 2002 Plant Mol. Biol.
Drought screening at reproductive stage at IRRI
Lafitte *et al.* 2004 TAG

Drought screening at reproductive stage at Coimbatore
Gomez *et al.* 2006 Am. J. Biochem Biotech
An F₆ mapping population

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Believe it or not, QTLs are accurate!

Adam H. Price

School of Biological Sciences, University of Aberdeen, Aberdeen, UK AB24 3UU

It is generally believed that mapping quantitative trait loci (QTLs) does not accurately position genes underlying polygenic traits on the genome, which limits the application of QTL analysis in marker-assisted selection and gene discovery. However, now that a few plant QTLs have been cloned or accurately tagged, it appears that they might be accurate to within 2 cM or less. This means that there will be circumstances when map-based cloning using only original mapping data would be a realistic option that avoids time-consuming and expensive fine mapping. Acceptance of this view would enhance the value of past and future mapping experiments, particularly those revealing small and environmentally sensitive QTLs that are often considered intractable at the molecular level.
## Early cloned or tagged QTLs in plants and original position

<table>
<thead>
<tr>
<th>Species</th>
<th>Trait</th>
<th>Gene/tagged locus</th>
<th>Mapping Population$^1$</th>
<th>Distance to original LOD peak (cM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major QTLs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>Fruit size</td>
<td>fw2.2</td>
<td>264 BC$_1$S</td>
<td>&lt;1.6</td>
<td>[9,10]</td>
</tr>
<tr>
<td>Tomato</td>
<td>Fruit shape</td>
<td>Ovate</td>
<td>82 F$_2$S</td>
<td>0</td>
<td>[11,12]</td>
</tr>
<tr>
<td>Arabidopsis</td>
<td>Flowering time</td>
<td>FLW1</td>
<td>98 RILs</td>
<td>0</td>
<td>[13]</td>
</tr>
<tr>
<td>Arabidopsis</td>
<td>Flowering time</td>
<td>CRY2</td>
<td>162 RILs</td>
<td>0.1 *</td>
<td>[14,15]</td>
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<tr>
<td>Arabidopsis</td>
<td>Transpiration</td>
<td>ERECTA</td>
<td>100 RILs</td>
<td>&lt; 1</td>
<td>[16]</td>
</tr>
<tr>
<td>Wheat</td>
<td>Frost tolerance</td>
<td>Cbf3</td>
<td>74 RILs</td>
<td>0.1 *</td>
<td>[17]</td>
</tr>
<tr>
<td>Wheat</td>
<td>Grain Protein</td>
<td>GPC</td>
<td>85 RICLs</td>
<td>0.2</td>
<td>[18,19]</td>
</tr>
<tr>
<td>Barley</td>
<td>Photoperiod response</td>
<td>Ppd-H1</td>
<td>94 DH</td>
<td>1.9</td>
<td>[20-22]</td>
</tr>
<tr>
<td>Soybean</td>
<td>Flowering time</td>
<td>FT1</td>
<td>156 RILs</td>
<td>0.4</td>
<td>[23,24]</td>
</tr>
<tr>
<td>Brassica</td>
<td>Flowering time</td>
<td>COL1</td>
<td>88 BC$_1$S</td>
<td>1</td>
<td>[25,26]</td>
</tr>
<tr>
<td>Brassica</td>
<td>Euric acid content</td>
<td>E1</td>
<td>184 F$_2$S</td>
<td>1</td>
<td>[27]</td>
</tr>
<tr>
<td><strong>Small QTLs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Maize x teosinte</td>
<td>Shoot morphology</td>
<td>tb1</td>
<td>290 F$_2$S</td>
<td>0.6 *</td>
<td>[28-30]</td>
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<tr>
<td>Rice</td>
<td>Heading date</td>
<td>Hd1</td>
<td>186 F$_2$S</td>
<td>0.5</td>
<td>[31,33]</td>
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<tr>
<td>Rice</td>
<td>&quot;</td>
<td>Hd2</td>
<td>186 F$_2$S</td>
<td>0.3</td>
<td>&quot;</td>
</tr>
<tr>
<td>Rice</td>
<td>&quot;</td>
<td>Hd3</td>
<td>186 F$_2$S</td>
<td>0</td>
<td>&quot;</td>
</tr>
<tr>
<td>Rice</td>
<td>&quot;</td>
<td>Hd4</td>
<td>186 F$_2$S</td>
<td>2.6</td>
<td>[32,30]</td>
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<tr>
<td>Rice</td>
<td>&quot;</td>
<td>Hd5</td>
<td>186 F$_2$S</td>
<td>1.2</td>
<td>&quot;</td>
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<tr>
<td>Rice</td>
<td>P uptake</td>
<td>Pup1</td>
<td>98 BILs</td>
<td>1</td>
<td>[34,35]</td>
</tr>
<tr>
<td>Rice</td>
<td>Grain weight</td>
<td>gw3.1</td>
<td>258 BC$_2$F$_2$S</td>
<td>&lt;1.6</td>
<td>[36,37]</td>
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<tr>
<td>Potato</td>
<td>Sugar content</td>
<td>inv/GE</td>
<td>146 F$_1$S</td>
<td>&lt; 3</td>
<td>[38,39]</td>
</tr>
</tbody>
</table>
A meta-analysis of drought and root related QTLS
### Meta-analysis with BioMercator-Chromosome 9

<table>
<thead>
<tr>
<th>AIC value</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model N</th>
</tr>
</thead>
<tbody>
<tr>
<td>633.98</td>
<td>637.67</td>
<td>688.52</td>
<td>663.71</td>
<td>659.14</td>
<td></td>
</tr>
<tr>
<td>Mean position 1 (C.I.)</td>
<td>77.69 (78.17-79.8)</td>
<td>59.3 (56.77-62.83)</td>
<td>56.14 (53.79-58.97)</td>
<td>21.32 (18.42-24.17)</td>
<td></td>
</tr>
<tr>
<td>Mean position 2 (C.I.)</td>
<td>63.62 (61.15-66.08)</td>
<td>70.18 (67.05-73.43)</td>
<td>61.66 (59.03-64.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean position 3 (C.I.)</td>
<td>81.66 (79.18-84.16)</td>
<td>99.42 (96.04-103.10)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean position 4 (C.I.)</td>
<td>(95.86-103.10)</td>
<td>(99.42-103.10)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Meta-analysis with BioMercator-Chromosome 9

Genes in confidence interval

- 15 Known function / motif
- 19 Expressed protein
- 47 Hypothetical protein
Eliminate candidates by expression analysis
Hard wax layer

1. Before hard layer
2. At hard layer
3. Buckled at hard layer

3 biological replications from 6 experiments

Affymetrix rice gene chip

52,000 transcripts
Number of genes in candidate region

81

Genes with Affymetrix probe set

No

14

Yes

67

Genes expressed

No

19

Yes

50

Number of genes sequenced to date

13
Allelic diversity detected

Expression polymorphism- 2 genes

Rate of genes with;
Nucleotide polymorphism Azucena- Bala 89%
Amino acid polymorphism Azucena-Bala 57%

Genetic diversity in rice
**Auxin transporters are candidates because:**

There are Aux or PIN transporters under the biggest QTLs

They have amino-acid polymorphisms

AUX responsive genes differ in expression between Bala and Azucena more frequently than expected.

Most root growth QTLs are QTLs for a-gravitropic phenotype

There is an Auxin by genotype interaction on root growth
### Meta-analysis of drought avoidance on chromosome 7

4 PIPs (aquaporins) here

<table>
<thead>
<tr>
<th></th>
<th>AIC value</th>
<th>Mean position 1 (C.I.)</th>
<th>Mean position 2 (C.I.)</th>
<th>Mean position 3 (C.I.)</th>
<th>Mean position 4 (C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>142.4</td>
<td>50.45 (45.54-55.36)</td>
<td>38.93 (29.8-48.07)</td>
<td>38.93 (29.8-48.07)</td>
<td>62.52 (49.82-75.22)</td>
<td>62.52 (49.82-75.22)</td>
</tr>
<tr>
<td>158.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>172.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>184.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Hydraulic conductance to test PIP candidacy

Experiment 1

Hydraulic conductance

Azucena NIL

Bala NIL

Experiment 2

Hydraulic conductance

Watered  Droughted
Hydraulic conductance of root pieces by osmotic swelling

Superglue

2 cm

1 M sorbitol 30 min

Water
Plasmolysis (1 M D-sorbitol and 1 mM CaCl₂) and swelling (distilled water) of root tips of RINILs different at QTL on chromosome 7
Both RINILs grown in same pot containing fine sand under flooded conditions for 2.5 weeks as control and droughted for 2 days after two weeks

Control

Drought
http://microrna.sanger.ac.uk


(miRNA detected from rice seedlings: a) non-treated as control, b) treated with 150 mM NaCl for 24 h for salt stress, and c) dehydrated for 12 h for drought stress)
TIGR Rice Loci
LOC_0s07g26590
aquaporin PIP2.1, putative, expressed

TIGR Rice Gene Models
LOC_0s07g26590.1

Azucena allele
Bala allele /93-11 allele

LOC_90_3'UTR Nip
osamiR1436

241 bp INDEL

AAGCATCGATGCCCGCTCTCTATCTGGAATATACTCCCTCGTCCCAAAATAAAGTGCA
----------------------------------ACTCCCTCGTCCCAAAATAAAGTGCA-----
-----------------------------ACTCCCTCGTCCCAAAATAAAGTGCA-----

Ba Az
95% confidence intervals for QTLs on bottom of Chromosome 1

sd1- semi-dwarf locus
Gibberellin 20 oxidase

Drought avoidance
Plant height
Plant mass
Root traits
Leaf Morphology
An F₆ mapping population

- Drought Screening
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Recombinant Inbred Near Isogenic Lines
Recombinant Inbred Near Isogenic Lines
Chromosome 9 NILs

Rhizotrons

Az allele

Ba allele

Pots

Az allele

Ba allele

![Graph showing Max. Visible Angle over Weeks 5 and 7 for Azucena and Bala alleles.](image)
Root density in field at IRRI (Jill Cairns)

KIII x Az NILs
Ba x Az NILs

Maximum root length in tubes
Prospects

It can be done

Tools for the physiology and theory

QTL data are a phenomenal resource

Root genes
  Mutants
  Allelic variation

Gene functions
  Transcriptomics and proteomic
Challenges and solutions

Root phenotyping
• Component traits => physiology and modelling
• High throughput screening => clever ideas and money

Plant-microbe interactions
• We know very little
Mycorrhiza

Looks like genetic variation exists
Challenges and solutions

Root phenotyping
• Component traits => physiology and modelling
• High throughput screening => clever ideas and money

Plant-microbe interactions
• We know very little

Linking to crop performance
• Quality field trials => know the soil
  => rainout shelters
  => avoid variation in phenology
  => replication in space and time
Soil physical properties are not well studied

Penetration Resistance Survey of WARDA
Showing Depth to 3 MPa Penetration Resistance

Above, high impeding field used in drought screens
Below, adjacent, lower impeding field

3MPa reached
0-10 cm
10-20 cm
20-30 cm
30-40 cm
40-50 cm
Not reached in 60 cm
Challenges and solutions

Root phenotyping
• Component traits => physiology and modelling
• High throughput screening => clever ideas and money

Plant-microbe interactions
• We know very little

Linking to crop performance
• Quality field trials => know the soil
  => rainout shelters
  => avoid variation in phenology
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Challenges and solutions

Allelic variation and capacity building

• Have we got all the diversity => multiple populations
  => association populations
Rice Diversity Project
Susan McCouch, Cornell

372 rice cultivars
82 different countries
75% now flowering

Allele testing
Histogram of Days to flower

21/371 yet to flower

<table>
<thead>
<tr>
<th>Rice subgroup</th>
<th>MicroRNA site present</th>
<th>MicroRNA site deleted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tropical japonicas</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>Temperate japonicas</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Indicas</td>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>106</strong></td>
<td><strong>230</strong></td>
</tr>
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</table>
Challenges and solutions

Allelic variation and capacity building
• Have we got all the diversity => multiple populations => association populations
• Can we exploit local selection pressure? => regional projects on regional germplasm

Genetics
• How do we handle epitasis? => big populations and stats?
Effect of 1 ppm Arsenate on Azucena and Bala

Control  AsO₄

Azucena

Bala
Tolerance of F₆ population to 1 ppm AsO₄

Susceptible

Tolerant

Azucena

Bala

% Root Length Class

Frequency
## Data linking arsenate tolerance to 3 genes

<table>
<thead>
<tr>
<th>Genotype at marker#</th>
<th>n</th>
<th>Arsenate tolerance (%) (± sd)</th>
<th>Sensitive/Tolerance score (0 or 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZ516 (6;10.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RG213 (6;~33.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RG257 (10;21.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>24.2 ± 3.3</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>24.2 ± 3.3</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>26.1 ± 2.4</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>23.7 ± 4.1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>49.3 ± 8.2</td>
<td>0.92 ± 0.29</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>23.2 ± 2.3</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>53.7 ± 6.0</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>54.9 ± 6.1</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>53.4 ± 6.1</td>
<td>1 ± 0</td>
</tr>
</tbody>
</table>
Challenges and solutions

Allelic variation and capacity building
• Have we got all the diversity => multiple populations
  => association populations
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Genetics
• How do we handle epitasis? => big populations and stats?

Candidate genes for sequenced species
• Fine mapping vs metaanalysis => knowledge of gene function
• Transcriptional regulation => cheaper arrays?
• Genetic polymorphism => cheap resequencing
• Posttranscriptional regulation => proteomics
• Proof of candidacy => easier transformation
Steve Briggs- San Diego

Peptide sequencing *Arabidopsis*

60,000 peptides
Big mistakes in annotation
396 novel genes
  179 are frame shifted
  217 are outside annotated genes
Correlation with transcription $r = 0.6$ i.e. 36%
Challenges and solutions

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Challenges and solutions

Bioinformatics
• Can we use data effectively? => Gramene, OryGenesDB

Impact and capacity building
• Can a GM approach be accepted? => ambassadors
• Have we got the pipeline to farmers? => capacity building
• Can we make the finances of collaboration easier?

People
• How do we get the most out of people => collaborate
• Is science an interesting career? => who do we want?
• How can we transfer knowledge and facilities to Africa?
Thanks