Gene hunting at the Carlsberg Laboratory employing historic barley mutants

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Barley mutants

- Short culm
- Early maturity
- Chlorophyll biosynthesis
- Wax biosynthesis
- Starch metabolism
- Barley malt quality
Why study short-culm mutants?

Global climate warming - higher temperatures and more extreme weather

Porter & Semenov 2005
Thunderstorms leads to lodging

Lodging

Retain humidity → fungal infections and pre-harvest germination

- Low yield
- Quality
- Mycotoxins
Short-culm plants

More resistant to lodging
Barley short-culm mutants

World wide short-culm barley collection (>1000 mutants) induced by

- M. Hayes, OR, USA
- E. I. Kivi, Finland
- A. Kleinhofs, WA, USA
- T. Konishi, Japan
- H. E. B. Larsson, Sweden
- L. Lehmann, Svalöv, Sweden
- M. Maluszynski, Vienna, Austria
- N. Mersinkov, Bulgaria
- R. T. Ramage, AZ, USA
- H. Ahokas, Finland
- T. K. Blake, Montana, USA
- H. Bockelman, Aberdeen, USA
- R. P. Ellis, Scotland, UK
- D. E. Falk, Canada
- D. C. Rasmusson, MN, USA
- F. Scholz, Gatersleben, Germany
- T. Tsuchiya, CO, USA
- S. E. Ullrich, WA, USA
- M. Vasa, Czechoslovakia
- D. M. Wesenberg, ID, USA
- R. I. Wolfe, Alberta, Canada
- U. Lundqvist, Svalöv, Sweden
- A. Faue, Fargo, USA
- G. Fischbeck, Weihenstephan, Germany
- H. P. K. Gaul, Grunbach, Germany
- P. R. Hanson, Cambridge, England
- E. A. Hockett, MT, USA
Different names to similar mutants

Historic: main mutant groups that include plants with reduced height

- Breviaristatum
- Brachytic
- Semi-brachytic
- Erectoides
- Dense spike
- Semidwarf

Different mother cultivars - comparision of phenotype difficult
Near Isogenic Lines

- 979 mutants (120 short-culm mutants)
- Cultivar Bowman
- Up to back cross 6

Udda Lundqvist and Jerry Franckowiak at 7th International BGS symposium, Saskatoon, August 1996
NILs with a short-culm phenotype

Main groups with barley short-culm mutants

Bowman, Brachytic, Semi-brachytic, Erectoides, Breviaristatum, Dense spike, Semidwarf

Near Isogenic Lines – comparison of phenotype possible
**uzu1.a**

- Mutant described already 1922 (Miyake and Imai 1922).
- Widely used in barley cultivars in Japan and South Korea
- *Uzu1* is orthologous to Arabidopsis *BRI1* (the brassinosteroid receptor)

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**Hormone class of brassinosteroids (BR)**

- discovered in 1979
- most active form brassinolide
- cell division
- stem elongation

![Brassinolide](image)
Christoph Dockter
uzu1.a – plant phenotype
23 brassinosteroid related mutants

ari.256  ert-ii  uzu  ert-a

ari.245  ari-b  ari-l  brh2  ari-o  brh.af  brh14  ert-u  ert-zd  ari-r  brh13  brh18  brh.i  ert-i  brh3.g  brh3.h  brh3.y  ert.z  slld5
Identification of brassinosteroid signaling mutants

Leaf unrolling bioassay (Honda et al., 2003)

Bowman

Bowman water

Bowman 20nM BL

Bowman 200nM BL

usu

BW885 water

BW885 20nM BL

BW885 200nM BL

sensitive

insensitive
23 brassinosteroid related mutants

4 brassinosteroid signaling mutants
- ari.256
- ert-ii
- uzu
- ert-a

19 brassinosteroid biosynthetic mutants
- ari.245
- ari-b
- ari-l
- brh2
- ari-o
- brh.af
- brh14
- ert-u
- ert-zd
- ari-r
- brh13
- brh18
- brh.i
- ert-l
- brh3.g
- brh3.h
- brh3.y
- ert-z
- sld5
In silico mapping approach

Druka et al. 2011, Plant Physiol. 155: 617-627

Determined the introgression region of ~900 of the 979 NILs!

“Genome Zipper”
In silico mapping approach

1. Where is the gene located?

2. Which mutants have an introgression region overlapping the gene location?
In silico mapping approach

<table>
<thead>
<tr>
<th>SNP introgression</th>
<th>barley genome zipper</th>
<th>barley genome map</th>
<th>candidate plants</th>
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<td>BW033</td>
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<td>865 860 912</td>
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</table>

BW033 (ari.256) Arg564 > Trp
BW312 (ert-ii.79) Thr573 > Lys

46 other NILs
Brassinosteroid receptor = *Uzu1* gene product

- 24 leucine-rich repeats (LRR)
- LRR6, LRR9 and LRR10 are missing in monocots

Island domain (ID) has the brassinosteroid binding site

TM = Transmembrane spanning domain

KD = Kinase domain

Modified residues in brassinosteroid binding site

PDB 3RGZ

Steric clashes or....
Modified residues in brassinosteroid binding site

... destroy the Bak1 co-receptor docking site?

Blue – charged residues
Red – hydrophobic residues
White – between blue and red
**uzu1.a causes modification in kinase domain**

Model of *HvBRI1* kinase domain based on template PDB-ID 2QKW_B

H857R causes steric clashes to F996 or interfere with binding to Bri1-binding partners.
**uzu1.a is temperature sensitivity**

14°C: weak phenotype

26°C: extreme dwarf phenotype like BRI1 knock-out in rice
Plants grown at 26°C get long new tillers when moved to 14°C.
**uzu1.a is temperature sensitivity**

14°C: weak phenotype

26°C: extreme dwarf phenotype like *BRI1* knock-out in rice

ert-ii.79 always strong
ari.256 always medium
What about the biosynthetic mutants

<table>
<thead>
<tr>
<th>4 brassinosteroid signaling mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>ari.256   ert-ii      uzu      ert-a</td>
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<table>
<thead>
<tr>
<th>19 brassinosteroid biosynthetic mutants</th>
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<tbody>
<tr>
<td>ari.245   ari-b    ari-l    brh2   ari-o   brh.af  brh14   ert-u   ert-zd  ari-r   brh13  brh18  brh.l  ert-t  brh3.g  brh3.h  brh3.y  ert-z  sld5</td>
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What about the biosynthetic mutants

Brassinosteroid synthesis pathway in *A. thaliana*

Dr. Damian Gruszka,
Univ. of Silesia, Poland
Matching location of genes with location of mutations (introgression regions)
Brassinosteroid synthesis pathway in *A. thaliana*

Dr. Damian Gruszka, Univ. of Silesia, Poland
GenomeZipper to find **DWF1**

- **ari-o.40** (W391R)
- **brh.af** (>18 kb deleted, neutrons)
- **brh14.q** (L210F)
- **brh16.v** (1 bp deletion)
- **ert-u.56** (exon 1 deleted, X-ray)
- **ert-zd.159** (G177V)
GenomeZipper to find **DWF1**

 barley genome map

- **BW053:** ari-o.40 (W391R)
- **BW072:** ari-o.297 (allelic to **uzu**)
- **BW085:** ari-o.301 (allelic to **uzu**)
- **BW087:** ari-o.304 (?????)
- **BW325:** ari-o.306 (?????)
- **BW333:** ari-o.308 (?????)

**HvDIM/DWF1**

- **1_0547:** contig 44330 139.92
- **3_0166:** contig 48789 140.63
- **1_0174:** contig 48176 140.86
**BRD1**

Brassinosteroid synthesis pathway in *A. thaliana*

Dr. Damian Gruszka, Univ. of Silesia, Poland

HvBRD1 = BR6ox
Physical map to find HvBRD1

ari-u.245 (non-sense)

brh.3.g (non-sense)

brh3.h (non-sense)

brh3.i (non-sense)

brh3.y (1 bp deletion)

ert-t.55 (not found)

ert-t.437 (1 bp deletion)
Physical map to find *HvBRD1*

**Physical Map**

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**Markers:**

- **BW031 ari-u.245** (non-sense)
- **BW091 brh3.g** (non-sense)
- **BW092 brh3.h** (non-sense)
- **BW094 brh3.y** (1 bp deletion)
- **BW324 ert-t.55** (not found)
- **BW110 ert-t.437** (1 bp deletion)
Physical map to find *HvBRD1*

- **ari-u.245** (non-sense)
- **brh.3.g** (non-sense)
- **brh3.h** (non-sense)
- **brh3.i** (non-sense)
- **brh3.y** (1 bp deletion)
- **ert-t.55** (not found)
- **ert-t.437** (1 bp deletion)
Physical map to find **HvBRD1**

**ari-u.245** (non-sense)

**brh3.g** (non-sense)

**brh3.h** (non-sense)

**brh3.i** (non-sense)

**brh3.y** (1 bp deletion)

**ert-t.55** (not found)

**ert-t.437** (1 bp deletion)
CPD is a redox enzyme that introduces a –OH group.

Brassinosteroid synthesis pathway in A. thaliana

Dr. Damian Gruszka, Univ. of Silesia, Poland
Physical map to find \textit{HvCPD}

<table>
<thead>
<tr>
<th>POPA SNP</th>
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<td>contig_47202</td>
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<td>MLOC_82054</td>
<td>44.24</td>
</tr>
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</table>

\textit{HvCPD}

- MLOC_10658

\textit{HvLEU}

- 2_1121 MLOC_34809 52.29

\textit{brh.13.p} (P445L)

\textit{brh18.ac} (P479S)
Looking for brassinosteroid deficient phenotypes in original mutants

TILLING mutant population (M2 population of N-methyl-N-nitrosourea treated plants of the barley double-haploid line H930-36)

16 short-culm mutants out of 950 plants

1 had *uzu* phenotype (including leaf unrolling)

950 sequenced for *Uzu* gene

*uzu1.c* (R710K)

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Dr. Damian Gruszka, Univ. of Silesia, Poland
Summary

- Brassinosteroid deficient phenotype identified
- 23 brassinosteroid mutants identified (4 signaling + 19 biosynthetic)
- *erectoides* (*ert*), *breviaristatum* (*ari*), *brachytic* (*brh*), *semi-dwarf* (*sdw*), *dense spike* (*dsp*) = can be the same
- 7 brassinosteroid biosynthetic genes and mutations in 3 of those
- 5 additional *uzu* alleles identified (*ert-ii.79, ari-245,uzu1.c, ari-o.297, ari-o.301*)
Thanks to...

- DFG
- Carlsberg Foundation
Bioinformatician wanted!

mats.hansson@carlsberglab.dk