



Public-Private Partnership in Pre-Breeding
Combining Knowledge from Field and Laboratory
for Pre-Breeding in Barley

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Ahmed Jahoor and Therese Bengtsson



PPP barley field trial, Iceland 2012. Photo: Magnus Göransson



WHAT IS PRE-BREEDING?

- Is the activities linking plant breeding research and plant genetic resources to development of adapted material.
 - Use of landraces, exotic material and wild relative of cultivated crops, carrying valuable genetic traits for future food and feed production.



URGENT NEED FOR PRE-BREEDING

- Varieties are not only grown today but represent the base for food production for the future.
 - Future climate changes
 - Emerging new diseases
 - Reducing environmental impact of agriculture and food production
- The existing genetic diversity in European plant breeding material might not be adequate to cope.
- A gap between applied research and basic research
 - Difficult to employ the outcome of basic science directly in practical plant breeding

SPECIFIC CHALLENGES OF CEREALS

- Maintaining high productivity and high quality with less input factors, less application of fertilizer and plant protection.
- Improved resistance against emerging diseases such Fusarium Head Blight in wheat, barley and oats, Ramularia and Biopolaris in barley as well as new strain of rusts.
- Improved winter hardiness in winter cereals
- Developing and integrating genome wide selection and genomic selection tools in the breeding process

NORDIC BARLEY PRE-BREEDING CONSORTIUM

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(PPP I)

NORDIC PUBLIC-PRIVATE PARTNERSHIP IN PRE-BREEDING

Background:

Specific Nordic growing conditions and increased dependency on seeds from large multi-national breeding companies

Aim:

Combine research and pre-breeding to support Nordic plant breeding

- **Broadening of the genetic basis for plant breeding**, e.g. facilitate adaptation to climate changes, present and emerging diseases
- **Introduction of specific genes for resistance, qualities etc.**, in adapted genetic background
- **Development of new technologies** that could speed up the breeding process



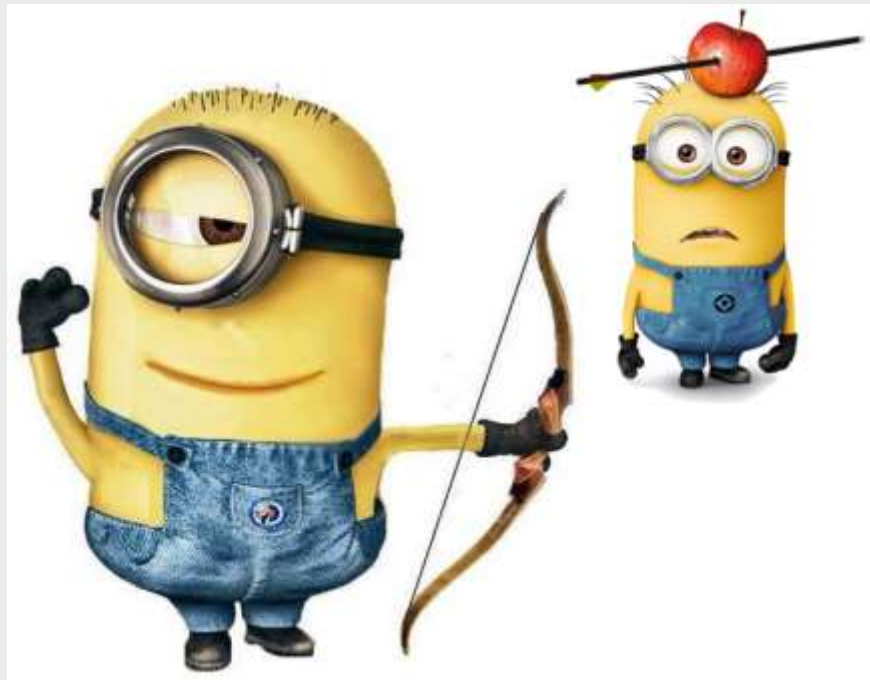
PROJECT GOALS, PPP I (2012-2014)

Foundation for effective cereal breeding for resistance and adaptation to changing climatic conditions :

- To develop and use available molecular markers to screen current breeding material
- To verify already published and available molecular markers in breeding material
- Identify linked DNA markers by association mapping for disease resistance and agronomic traits
- Assist in breeding of varieties with plasticity for a climate with more extreme weather conditions in Nordic Regions
- To educate PhD Students and Post Docs



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TRUST AND MUTUAL
UNDERSTANDING

**"RIGHT, NOW THAT A RELATIONSHIP
OF TRUST HAS BEEN ESTABLISHED,
LET'S GET DOWN TO BUSINESS,
SHALL WE?"**

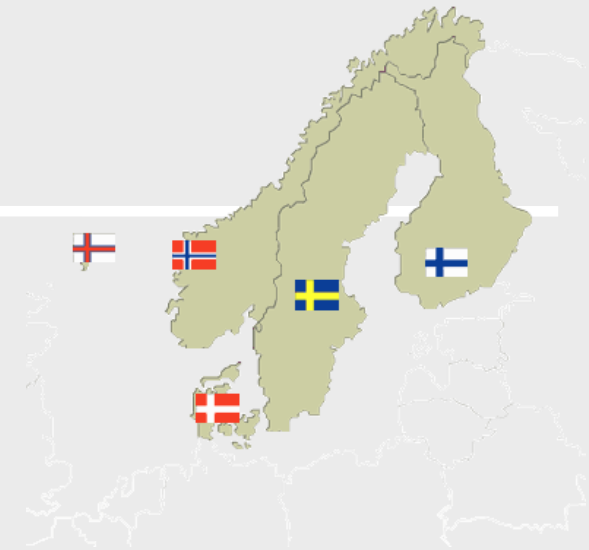
GENOTYPING - ASSOCIATION MAPPING PERIOD I (2012-2014)

- **Spring barley panel:** 180 advanced breeding lines and cultivars (**30 lines/breeder**)
- **48 SSR markers** spanning all chromosomes
- Barley iSelect **SNP chip** based on the Illumina Infinium assay (9K)
- 7842 high-confidence SNPs derived from expressed genes → **6208 polymorphic**



PHENOTYPING ACTIVITIES, PPP I

Disease traits	2012	2013	2014
Powdery Mildew	2	3	3
Bipolaris	1	1	1
Fusarium	1		1
Net Blotch Net Form	1	1	3
Net Blotch Spot Form	1	1	1
Nematode	1	1	
Ramularia	2	2	1
Scald	4	6	2
Leaf Rust			2
DON (ELISA)	3		
NIV (ELISA)	1		
Cleistogamy (KASP)			1
Total	17	15	15



Agronomic traits	2012	2013	2014
Development	11	13	7
Height	10	11	3
Straw strength	10	10	4
Yield	4	4	2
Total	35	38	16

Development: heading date, days from sowing to heading, days from sowing to ripening

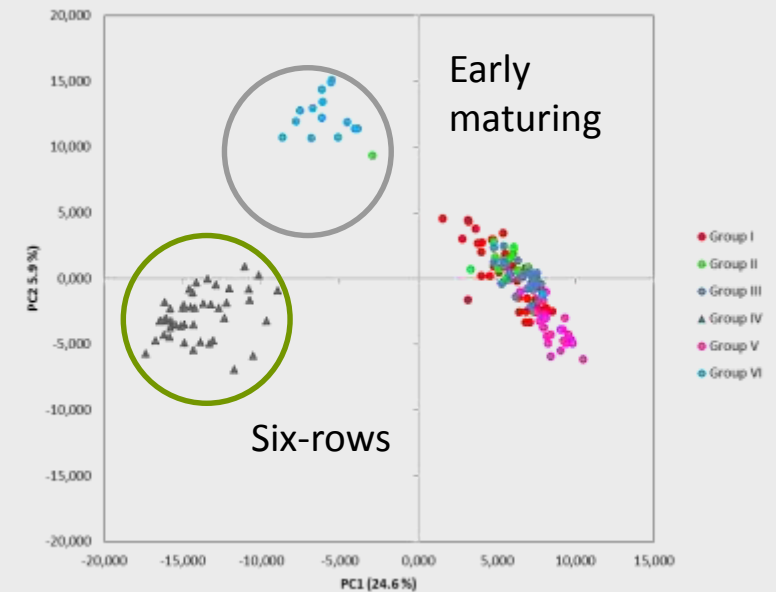
Straw strength: lodging, straw breaking, necking, shattering

DON (deoxynivalenol), NIV (nivalenol) - Fusarium toxins

POPULATION STRUCTURE & DIVERSITY IN THE BARLEY COLLECTION

- Six groups
- Row-type, geographical origin, earliness
- Most private alleles found in the six-rows

Group	Population size	No. of two-rows	No. of six-rows	Average number of alleles per locus	No of private alleles	Genetic Diversity \bar{H}	Standard deviation of \bar{H}
I	40	40	---	1.8	34	0.28	0.28
II	17	17	---	1.7	6	0.25	0.36
III	30	30	---	1.8	6	0.25	0.30
IV	45	---	45	1.8	447	0.23	0.23
V	22	22	---	1.7	3	0.20	0.37
VI	15	15	---	1.6	11	0.20	0.47
Total	169	124	45			0.36	0.45



Based on SNP data

LINKAGE DISEQUILIBRIUM (LD) DECAY IN THE BARLEY COLLECTION

250-500 markers needed to cover the barley genome

- A more rapid LD decay in the six-rows and lines from north (Group II, IV & VI)

- Overall rapid LD decay on:

2H (eg. *Vrs1*)

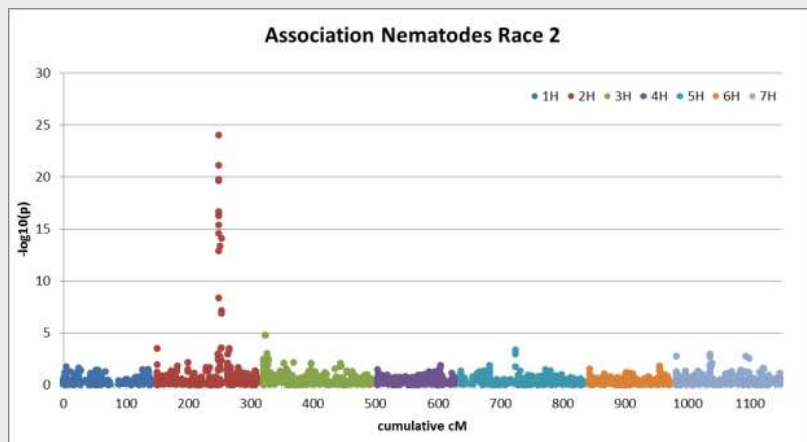
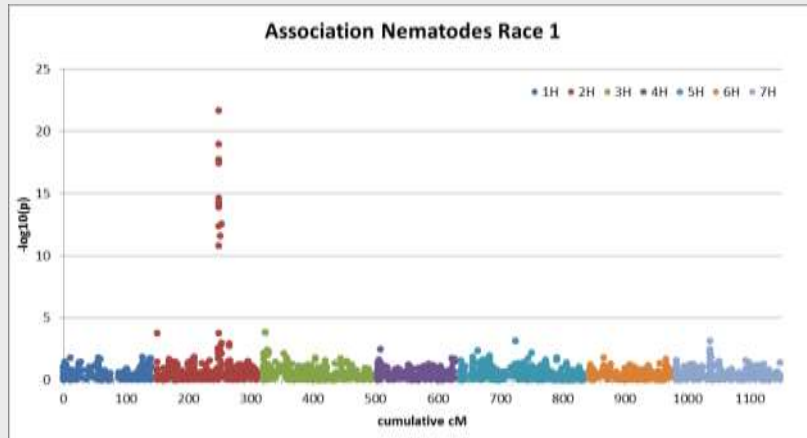
4H (eg. *mlo*, *sgH1*)

7H (*nud*, *sgH3*)

Chromosome	Total population	Two-rows	Six-rows	Group I	Group II	Group III	Group IV	Group V	Group VI
1H	0-3	5.5-9	0-3	2.5-6	0-3	2.5-6	0-3	2.5-6	0-3
2H	0-3.5	0-3.5	0-3.5	0-3.5	0-3.5	3-6.5	0-3.5	6-10	0-3.5
3H	3.5-7	10.5-14	0-3.5	7-10.5	3.5-7	0-3.5	0-3.5	3.5-7	0-3.5
4H	0-2.5	0-2.5	0-2.5	0-2.5	0-2.5	2.5-5	0-2.5	4.5-7.5	0-2.5
5H	0-4	0-4	4-8	0-4	0-4	0-3.5	4-8	8-12	0-4
6H	0-3	7.5-10.5	0-3	2.5-5.5	7.5-10.5	5-8	0-3	2.5-5.5	0-3
7H	0-3.5	0-3.5	0-3.5	2.5-5.5	0-3.5	0-3.5	0-3.5	0-3.5	---
Whole genome	0-4	4-8	0-4	2.5-6	0-4	4-8	0-4	4-8	0-4

Intra-chromosomal comparisons

EXAMPLE OF RESULT FROM THE GWAS ANALYSES



Cereal Cyst Nematode

- Two markers have been found on 2H
- Converted to KASP and currently in use by the companies
- Haplotype AA almost full resistant
- Only AA haplotype in Denmark
- Allele C for marker 1- susceptible

Marker 1	Marker 2	# of lines	Ave. CCN infection
A	A	71	1.2
A	G	17	6.6
C	A	51	6.7
C	G	37	6.4



MARKERS FOUND, PPP I

Trait	Chromosome	Markers converted to KASP (2014)
Powdery mildew	4H	Yes
Powdery mildew	6H	Yes
Nematode	2H	Yes
Scald	3H	
Scald	7H	Yes
Heading date, maturity and plant height	3H	Yes
Plant height	1H	
Plant height	7H	
Earliness	2H	Yes
Straw breaking	2H	
Straw breaking	2H	
Straw breaking	2H	
Straw breaking	4H	Yes
Fusarium	2H	
Fusarium	5H	
Ramularia	4H	
Spot blotch	1H	
Spot blotch	7H	
Row-type	2H	Yes
Grain texture	5H	
Grain softness	5H	

- Found markers are/were converted to KASP genotyping markers
- Validated in breeding material

PROJECT GOALS, PPP II (2015-2017)

Foundation for effective cereal breeding for resistance and adaptation to changing climatic conditions :

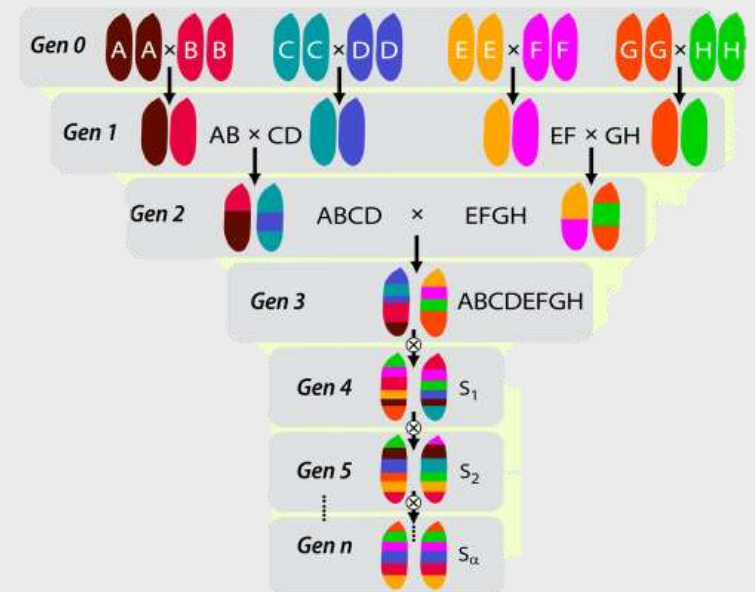
- To screen current breeding material as well as new material to find a larger variability of genes for abiotic and biotic stresses with emphasis on various climatic conditions
- To introduce these genes in relevant breeding material by using MAGIC populations
- Identify linked DNA markers by association mapping for disease resistance and agronomic traits for climate changes
- To develop DNA markers for the found traits to be used by the breeders
- To educate PhD Students and Post Docs

WORK PACKAGES IN PPP PROJECT PHASE II:

1. Development of Multiparent Advanced Generation InterCross (MAGIC) populations
2. Development of homozygous lines from Magic populations
3. Searching for and screening of new sources of disease resistance
4. Screening of segregation populations for diseases resistance
5. Screening of segregating populations for agronomic traits
6. Data management and association mapping
7. Transferability and usefulness of new resistance and agronomic trait genes in different germplasm

MULTI-PARENT ADVANCED GENERATION INTER-CROSS (MAGIC) POPULATIONS (1)

- Development of MAGIC populations
- Single line- resistant to several diseases etc.
- Overcome problems with bi-parental populations
- Segregating populations increase allele frequencies of rare alleles



Bandillo et al. Multi-parent advanced generation inter-cross (MAGIC) populations in rice: Progress and potential for genetics research and breeding. RICE 6(1), 2013

CROSSES, DH PRODUCTION AND MULTIPLICATION ACTIVITIES (1&2)

- **Six MAGIC populations** (for resistance traits) are developed and DH production completed for four and in progress for two
- Development of **an 8-way MAGIC population for early flowering/ early maturing** Crosses have been made and DH produced and DH lines are in the multiplication in Denmark and Finland
- 2016/2017- Multiplication of DH lines at New Zealand was carried out for resistance MAGICS
- Ongoing- Screening of new resistance sources for new crosses



SCREENING OF THE MAGIC POPULATIONS

Pyramided traits	No. of MAGIC populations	Parents per population	Individuals per population	Location of screening (2017):
Scald, Rust, Net Blotch Spot Type	2	8	122; 29	LUKE –STNB (all) LBHI-scaId (29) NOS & Sejet- Scald (all) NOS & Sejet- Rust (all)
Scald, Fusarium	1	8	81	Graminor & LUKE- Fusarium (51) LBHI-scaId (81) NOS & Sejet- Scald (all) NOS & Sejet- Rust (all)
Overall resistance, Scald, Fusarium	1	8	303	Graminor & LUKE- Fusarium (128) LUKE- Scald (303) NOS & Sejet- Scald (all) NOS & Sejet- Rust (all)
Earliness	1	8	400	Boreal- earliness (all)
Rust	2	4	90; 96	NOS & Sejet (all)

DATA MANAGEMENT AND ASSOCIATION MAPPING (6)

- All information and results are made available for the partners via the internal CMS that was built up during PPP I
- Collecting the material from the MAGIC populations produced within the project and isolate DNA for genotyping of high throughput Single Nucleotide Polymorphism
- Association between the field data from WP3, WP4 and WP5 and the marker data with Genome Wide Association Analysis (GWAS)

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The Project Plant Breeding Entities

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