A large scale association study for aroma wheat compounds in bread wheat

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Outline of the presentation

- Plant Material
- Diversity of the wheat collection used.
- Genome wide association
- In addition to the association results three questions will be answered:
  1. What was the effect of modern breeding on the genetic diversity of European wheat?
  2. What was the effect of modern breeding on the allele frequencies?
  3. Are the aroma compounds same in landraces/old cultivars and new cultivars?
Plant Materials

• 192 accessions.
• From 14 countries.
• *Triticum aestivum*,
• Cultivars and landraces
  • Landraces (39 accessions)
  • 1886-1940 (16 accessions)
  • 1940-1960 (12 accessions)
  • 1960-1980 (14 accessions)
  • 1980-2000 (40 accessions)
  • 2000-2009 (64 accessions)
• Winter (156) and spring (36)
Genotyping and phenotyping of the plant material

- The plant collection was genotyped with two DNA molecular marker systems:
  - Simple Sequence Repeats (SSR) markers
  - Diversity Array Technology (DArT) markers
- A total of 76 aromatic compounds were analyzed using Dynamic Headspace Extraction and Gas Chromatography Mass Spectrometry.
SSR markers

All 76 SSR markers were polymorphic.

Allele number per marker ranged from 2 to 27 alleles.

Polymorphism Information Content (PIC) for the SSR markers ranged from 0.04 to 0.9.

### SRR marker distribution across the wheat genomes

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<th>Chrom</th>
<th>SSR loci</th>
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DArT markers

- 188 accessions were genotyped with Wheat DArT PstI(TaqI) v3 (7000 markers)
- 2532 polymorphic markeres
- Reproducibility 97-100% (how reproducible the scoring for replicated samples is)
- Call Rate 80.5-100 %. (valid scores in all possible scores for a marker)
- PIC 0.011-0.5

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<th>DArT loci</th>
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<td>Total</td>
<td>2532</td>
<td>533</td>
<td>873</td>
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Mantel test showing the correlation between modified Roger’s distance and Jaccard’s distances resulted from SSRs and DArTs markers, respectively.

Pearson's r: 0.66
PCA of AROMA Wheat Accessions Grouped by Date and Verbalization type Based on SSR Markers

Dimension 1 vs Dimension 2

Legend:
- Spring:Landrace
- Spring:<1940
- Spring:1940-1960
- Spring:1960-1980
- Spring:1980-2000
- Spring:2000-2009
- Winter:Landrace
- Winter:<1940
- Winter:1940-1960
- Winter:1960-1980
PCA of AROMA Wheat Accessions Grouped by Date and Verbalization type Based on DArT Markers
PCA of AROMA Wheat Accessions Grouped by Date and Verbalization type Based on DArT Markers

**DArT**

**SSR**
Wheat genetic diversity in Changes Europe over time (20-year interval) based on SSR and DArT

- The period from 1960 to 1980 was coincident in Europe and also worldwide with two major events:
  1. the introduction of the semi dwarf genes *RthB1b* (*Rht1*) and *Rht-D1b* (*Rht2*)
  2. the incorporation of the 1RS-1BL wheat-rye translocation.
  3. the intensive use of a small number of varieties as genitors in the different European breeding programs.
Why to analyze the changes in allele frequencies?

- Over time, alleles frequency is changing in favour of the allele(s) conferring the desirable trait expression

Example

<table>
<thead>
<tr>
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<th>Population 1</th>
<th>Population 2</th>
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<td>Markere x</td>
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<td>A2</td>
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<td>%</td>
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</table>

Marker has the same PIC value in both populations and both populations has the same genetic diversity index $H$
Changes in allelic frequency over time in two SSR markers

- **WMS261** is closely linked to dwarfing gene *Rht8*.
- Has 21 different alleles.
- Lines carrying the allele (164) bp at the locus WMS 261 were significantly taller than lines carrying allele (174).

- **GWM344** is closely linked to powdery mildew resistance gene *Pm22*.
- Closely linked to the leaf rust resistance gene *Lr14a*.
- Associated with yellow pigment kernel content in durum wheat.
**Changes in allelic frequency over time in two DArT markers**

**wPt-743218**
- associated with the canopy temperature during the grain filling stage
- might be due to adaptation to climatic changes

**wPt-7946**
- co-segregating with wmc222 in, which is linked to two stem rust resistance genes \( sr33 \) and \( sr45 \)
- Might be due the negligence of stem rust association
Genome wide association mapping

- Association mapping is an linkage between two or more alleles at different loci via linkage disequilibrium or LD (non-random association of alleles at different loci).
- Aims to identify specific functional variants (i.e., loci, alleles) linked to phenotypic differences in a trait.
- No need to make crosses initially to generate segregating populations
- Utilize the natural variation that exists in the available germplasm.
Aroma compounds in bread wheat

- The aroma compounds were extracted from freshly milled whole wheat flour using dynamic headspace sampling.
- Volatiles were separated and identified using Gas Chromatography-Mass Spectrometry (GC-MS).
- A total of 76 volatile compounds were identified.
- The relative content of those compounds were represented by peak area and the values were log transformed to 1 to 9 scale.
Aroma compounds in bread wheat

- A total of 267 putative QTLs were found to be associated with 76 traits related to aroma.
- The putative QTLs $-\log_{10} P$ value ranged from 3 to 15.5.
- Two markers were found to be associated with several aroma compounds, the first marker
  - wPt-009958, which is located at the position 18.8 cM on the chromosome 2A and found to be associated with 23 traits.
  - wPt-008460 at the position 77 cM on chromosome 2B and found to associate with 19 traits
- The strongest association was found between the marker wPt-004398 and pAllylanisole with $-\log_{10} P$ value of 15.5
Genome wide association scan for $p$-allylanisole
Variation of 76 different volatile compounds among landraces/old cultivars and new cultivars

Measurements of all compounds were log transformed to 1 to 9 scale
Difference in aroma compounds between landraces/old cultivars and new cultivars
Top 10 loci that were found to be associated with 46 different volatile compounds.

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<tr>
<th>Loci</th>
<th>Ethyl Acetate</th>
<th>Dimethyl Sulfoxide</th>
<th>Gamma Butyrolactone</th>
<th>1-Hexyl acetate</th>
<th>2-Propyl Acetate</th>
<th>1-Octanol</th>
<th>2-Propanol</th>
<th>2-Butanol</th>
<th>2-Pentanone</th>
<th>4-Methyl Isoamyl Alcohol</th>
<th>Hexanal</th>
<th>Benzaldehyde</th>
<th>2-Heptanal</th>
<th>Pyrazine</th>
<th>2,3-Dimethyl Hexanol</th>
<th>1,8-Cineole</th>
<th>3-Octanone</th>
<th>1,9-Octadecenal</th>
<th>3-Cyclohexanone</th>
<th>1H-Pyrole</th>
<th>2-Heptanone</th>
<th>2-Pyridine</th>
<th>2-Dimethyl</th>
<th>Hexano</th>
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Take home message

- Modern wheat breeding in Europe did not cause a reduction in overall wheat genetic diversity.
- Separations based on vernalization type and releasing year were observed in bread wheat.
- Changes in allele frequencies were found in loci that closely linked to important traits.
- High variability in aroma compounds, which reflect the genetic richness of the plant collection.
- Differences in aroma compounds were observed among landraces/old cultivars and new cultivars.
- Several putative QTLs were mapped using association mapping that can be used to breed new cultivars with enhanced aroma compounds.
Acknowledgment

The Ministry of Food, Agriculture and Fisheries of Denmark.

NordGen

IPK
That was our catch from fishing in the wheat gene pool

Thanks for your attention