DEPARTMENT OF PLANT SCIENCE AND ENVIRONMENT
FACULTY OF SCIENCE
UNIVERSITY OF COPENHAGEN

Association Mapping in a Complex Synthetic and Common Hexaploid Wheat Collection Using DArT Markers.

Jihad Orabi[1, 2], Lars Eriksen[3], Jeppe R. Andersen[4], Lise N. Jorgensen[5], Ahmed Jahoor[4, 6] and Gunter Backes[1, 7]

1- Department of plant and Agricultural sciences, Copenhagen University, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark. 2- Corresponding author email: jio@life.ku.dk. 3- Sejet Planteforædling, Nørremarksvej 67, 8700 Horsens Denmark

4- Nordic Seed A/S, Højbygårdvej 14, 4960 Holeby, Denmark. 5- Department of Agroecology, Flakkebjerg, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark

6- Department of plant breeding, the Swedish University of Agricultural Sciences, 2353, Alnarp, Sweden. 7- Faculty of Organic Agriculture Sciences, University of Kassel, Nordbahnhofstr. 1a, D-37213 Witzenhausen, Germany

Abstract:
A collection of synthetic and common hexaploid wheat (*Triticum aestivum* L.) was used to study the association between major wheat diseases in Denmark and 1192 mapped polymorphic DArT markers. The 184 wheat lines collection composed of 122 synthetic wheat lines and 62 common wheat lines. Wheat diseases scored in three years and at three locations in field and semi-field experiments. Diseases included in this study are powdery mildew (*Blumeria graminis*); yellow rust (*Puccinia striiformis*); brown rust (*Puccinia triticina*), Fusarium head blight (*Fusarium* spp.) and mycotoxins (DON (Deoxynivalenol), NIV (Nivalenol) and ZEA (Zearalenone) produced by fusarium. A total of 14 independent disease/year traits were included in the association analysis and yielded more than 150 DArT markers with $-\log_{10} P$ score equal or higher than 3. However, the complex population structure of the collection made it difficult to separate the true from false positive association with the DArT markers. Therefore we examined selection criteria to screen the obtained QTL based on 4 magnitudes: 1) The LOD score value of the associated marker on the trait 2) The standard deviation of the effect, 3) having more than one DArT marker with relatively high LOD score flanking the DArT marker associated with the trait, 4) a clear difference in disease reaction between accessions that have "1" allele and accessions that have "0" allele over most plant groups (synthetic and common) aiming at avoiding the effect of genetic structure of the plant collection over the association. By applying these criteria we reduced the number of putative QTLs to 21 QTLs. Out of the 21 putative QTLs, 12 were found novel QTLs and 9 QTLs were positioned on the same chromosomal location of known QTLs/Genes, which validate the criteria we used to screen out the false positive QTLs.

Introduction:
One of the main constrains of bread wheat development is the narrow genetic background. Only few individuals during wheat hybridization and the domestication were involved, which caused a genetic bottleneck. Synthetic wheat, created by crossing tetraploid wheat (durum or emmer) as a donor of A and B genomes with wild progenitor (*Aegilops Tauschii*) as source of D geneome (Figure 1) is a donor of many diseases, abiotic stress and quality traits (Mujeeb-Kazi 2006; Dreisigacker et al. 2008).

Association mapping or linkage disequilibrium mapping is an linkage between two or more alleles at different loci. A large number of markers needed to discover the association in a population. Diversity array Technology (**DArT)** is a hybridization based technology that requires no prior knowledge of sequencing information. By scoring the presence versus absence of a specific DNA fragment in genomic DNA representations, which generated from samples of genomic DNA, hundreds to thousands of genomic loci can be typed in parallel. The technology was developed for first time in a diploid crop, (Jaccoud 2001). We aimed from this study to conduct a genome wide association between wheat major diseases in Denmark and a collection of normal and synthetic wheat accessions.

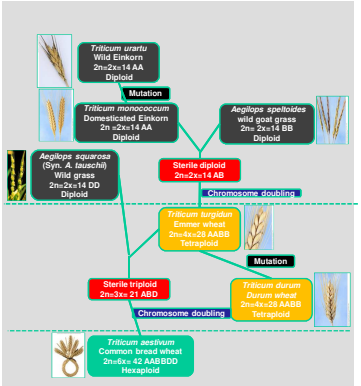

Figure 1: Origin of Hexaploid wheat

Materials and methods:

1 - Plant material
A total of 192 accessions was used in this study, of which 68 were common (hexaploid) wheat and 124 accessions were synthetic hexaploid wheat.

2 - SSR and DArT Markers
A total of 81 SSR markers (27 per geneome) and 1792 DArT markers were employed to study the genetic structure of the collection and to reveal the association between the accessions and several diseases.

3 - Disease assessment
The diseases evaluation was carried out during two seasons in field and semi-field experiment for several traits/disease at Sejet, Nordic Seed and Flakkebjerg. Several wheat diseases were included in the assessment (Powdery Mildew (Pm), Yellow Rust (Yr), Brown (Leaf) Rust (Br) and Fusarium Head Blight (Fu). In addition 3 toxines i.e Deoxynivalenol (Don), Zearalenone (Zea) and Nivalenol (Niv)(. Number of tested accessions varied due to the seed limitation and/or evaluation capacity

4 - Association study:
In the association study only the mapped DArT markers were included. A total of 1172 mapped DArT markers out of 1792 markers were found on a censuses map using 9 different mapping population produced by Triticatre (http://www.triticarte.com.au). These markers were distributed over all three wheat genome. A total of 433, 470 and 269 DArT markers were mapped to A, B and D genome, respectively. The remaining 620 DArT markers were not assigned to any chromosome, therefore they were excluded from the association study but they were included the genetic collection structure analysis.

Results and discussion
The association analysis yielded more than 150 DArT markers with $-\log_{10}$ (p) ≥ 3. However, the number of QTLs determined by these markers was reduced to 21 (Table 1) QTLs based on several criteria:

1. The $-\log_{10}$ (p) value.
2. The effect value.
3. The standard deviation of the effect.
4. Having more than one DArT marker with relatively high $-\log_{10}$ (p) score flanking the DArT marker associated with the trait.
5. Group (synthetic/non-synthetic wheat) independent association.

Out of 21 putative QTLs we found, 4 QTLs were found against powdery mildew disease, 5 against yellow rust, one against brown (leaf) rust and 11 against Fusarium head blight disease and toxins. The map positions of the associated DArT markers on comparative genetic map were used to compare the genetic position of each putative QTL found with the previously known QTL(s)\gene(s) at the same chromosomal position. The map comparison showed that 11 QTLs might be novel QTLs and have not been reported before (Table1).

An example of the map comparison is presented in figure 2 for QTL number 6 (Table 1) against yellow rust disease. This QTL was associated with wPt-0335 and mapped to chromosome 2B near or in a region that was reported to have a cluster of yellow rust genes and QTLs. Many resistance genes and QTLs against stripe rust disease have been mapped to chromosome 2B, including *Yr5*, *Yr7*, *Yr27*, *Yr31*, *YrQz*, *QYr3* and *YrZak* (Boukhatem et al.2002; Yan et al. 2003; Deng et al. 2004; MacDonald et al. 2004; Sui et al.2009) . All these genes were clustered in the proximal region on the short arm of wheat chromosome 2BS. *Yr27* was located on wheat chromosome arm 2BS, closely linked to leaf rust resistance genes *Lr13* and *Lr23* in the proximal region (McDonald et al 2004). *Yr31* also is located in or near a cluster of resistance genes in the proximal region on the short arm of wheat chromosome 2BS, and the genes located in the region include leaf rust resistance genes *Lr13* and *Lr23*, stripe rust resistance genes *YrSp* and *Yr31*, and stem rust resistance gene *Sr10*.

Table 1, the main putative QTLs found based on the association study and their best associated accessions

#	Trait	Marker	Chro.	Pos. cm	$-\log_{10} P$	Eff.	Best 3 Accessions	Co-localized with Known Gene/QTL
1	PmFb	wPt-664609	1D	66.9	4.9	2.3*	18,14,153	X
2	PmFb	wPt-4527	2B	33.8	3.4	9.5§	30,43,113	X
3	PmFb	wPt-0079	2B	81.3	3.3	10.2§	30,43,113	X
4	PmSj	wPt-3978	3A	204	3.5	1.2*	18,40,219	X
5	YrNs	wPt-5195	2B	9.3	3.2	1.5*	115,151,65	X
6	YrNs	wPt-0335	2B	76.0	3.1	1.4*	115,66,151	Yr5 2BL (Yan et al. 2003). Yr7 2BL (Bariana et al. 2001). YrQz 2B (Deng et al. 2004). QYR3 2BS (Boukhatem et al. 2002)
7	YrNs	wPt-0047	2B	112	3.2	1.6*	151,65,168	X
8	YrSj	wPt-8446	3B	11.4	8.9	2.7*	113,115,65	QTL 3BS (Suenaga et al. 2003). Yrns-B1 3BS (Börner et al 2000). QTL 3BS (Singh et al. 2000)
9	YrSj	wPt-669837	5B	43.8	3.2	2.4*	113,115,65	Yr19
10	BrNS	wPt-3697	3A	161	5.5	1.8*	113,115,219	QLr.sfr-3A (Messmer et al. 2000)
11	Don/NivFb	wPt-4107	1B	9.1	8/5.7	1.7/0.8 ‡	159,161,162	QTLs (Zhange et al. 2004; Lin et al. 2004; Schmolke et al. 2005, Klahr et al. 2007)
12	Don/NivFb	wPt-5587	2B	4.2	4/3.4	1.2/0.7	159,161,162	X
13	FuFb	wPt-9749	2D	90.5	3.1	29.7§	113,189,115	QFhs.crc-2D (Somers et al. 2003). QFhs.nau-2DL (Jiang et al. 2007)
14	Don-Niv-ZeaFB	wPt-9749	2D	90.5	3.8/3.2/5.7	1.2/0.6/0.7	159,161,162	
15	FuFb	wPt-6419	2D	48.6	3.2	2.9†	161,162,167	QFh.Pur.2D (Shen et al. 2003)
16	FuFb	wPt-006909	3D	151.8	5.05	5.3†	161,162,189	X
17	FuFb	wPt-006965	3D	11.03	4.37	4.6†	161,162,189	X
18	Don-Niv-ZeaFB	wPt-8650	4B	22.6	3/3.8/3.8	1/0.6/0.6	59,111,159	QTLs (Somers et al. 2003; Yang et al. 2005; Lin et al. 2006)
19	FuFb	wPt-669837	5B	43.80	3.00	5.03†	161,162,189	X
20	Don-Niv-ZeaFB	wPt-1951	5B	42.9	4.4/4.9/2.9	0.6/0.6/0.9‡	159,161,162	QTLs (Paillard et al. 2004, Yang et al. 2005, Jia et al. 2005, Häberle et al. 2009)
21	FuSf	wPt-3093	7B	201	4.2	46.8§	64,113,189	X

*Scale 1-9, † Scale Infected ears (out of 15), § Scale 1-99, ‡ Scale Log µg

Map comparison showed that our QTL associated with DArT marker wPt-0335 is localized among the cluster of resistance genes in the proximal region on the short arm of wheat chromosome 2BS (Figure 2). This results confirm our methodology and the other results obtained.

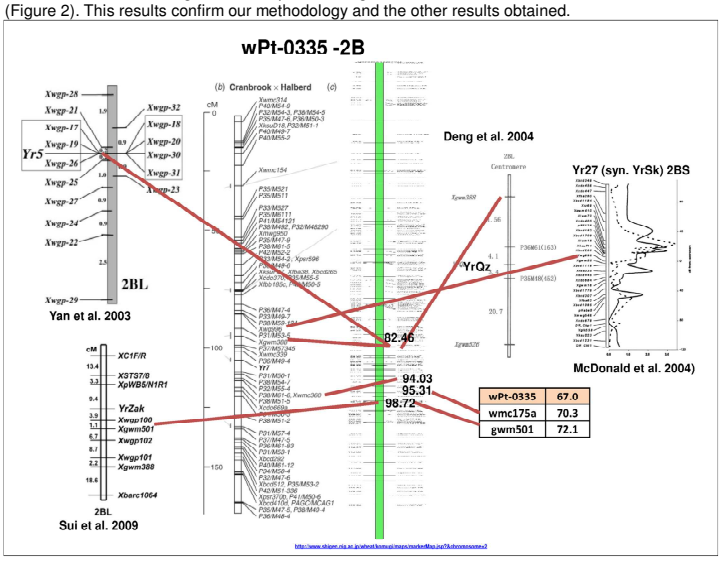

Figure 2. Map comparison for yellow rust genes and QTLs on chromosome 2 B

For each QTL, the best 3 accessions (most resistant\tolerant) carrying the right allele (1 or 0) according to the association analysis were pointed out and listed in table 1 in order to use them in the breeding program. These lines have been already crossed with advanced line cultivars and markers flanking their relevant QTL were employed for marker assisted breeding.

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