Sequence–based tools facilitate high-density mapping of a Russian wheat aphid resistance gene in wheat

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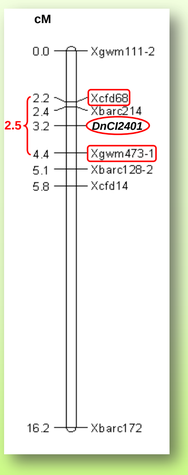
Background

Bread wheat (*Triticum aestivum* L.) is one of the most important crop species providing staple food for 35% of the world's population. It is an allohexaploid species (2n = 6x = 42, AABBDD genome) originating from two interspecific hybridizations. Genome mapping as well as positional cloning in bread wheat are hampered by its huge genome size (~ 17 Gbp), polyploidy and large amount of repetitive sequences (> 80%).

Russian wheat aphid (*Diuraphis noxia*), native in Afghanistan, is an important world invasive pest of wheat and barley crops. Several *D. noxia* biotypes with various virulency have spread in all wheat and barley growing areas with the exception of Australia. Host resistance is the most efficient, economical, and environmentally safe approach to protect wheat from pathogens while minimizing the use of pesticides. Several genes contributing to RWA resistance were identified in various wheat lines but only a few confer resistance to highly virulent US RWA biotype 2. CI2401 line carries *DnCI2401* gene that underlies resistance to RWA biotype 2 on the short arm of chromosome 7D (7DS).

Construction of a high-density genetic map covering the Dn*CI2401* gene region is essential for positional cloning of this resistance gene as well as marker-assisted selection. In this work, we present an approach to marker development employing new sequence-based tools and resources.

Genetic map



- Flanking SSR markers **cfd68** and **gwm473** delimit an interval of 2.5 cM around the resistance gene *DnCI2401*
- Saturation of genetic map in this region is essential for positional cloning of the *DnCI2401* gene

Mapping populations

- **CI2401 x Glupro**

  CI2401 – resistant – carrying ***DnCI2401***
  cv. Glupro – susceptible
  152 F2 plants

- **Synthetic W7984 x Opata 85 (ITMI)**

  Subsidiary population
  with a higher level of polymorphism
  115 F8 RILs

Bread wheat
(*Triticum aestivum*)

Russian wheat aphid
(*Diuraphis noxia*)

7AS, 7BS and 7DS Illumina paired-read sequence data

- Flow-sorted 7AS, 7BS and 7DS arms amplified by multiple displacement amplification have been sequenced by Illumina GAIIx technology
- Unique and low copy regions of the chromosome arms were assembled and scaffolded

Chromosome arm	Length (Mbp)	Sequence data (Mbp)	Coverage
7AS	407	8,700	21,3x
7BS	360	10,800	30x
7DS	381	13,050	34,2x

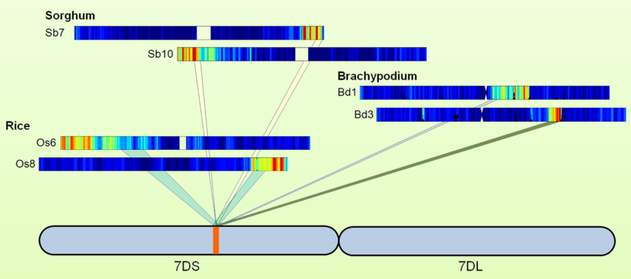
Draft assemblies are publicaly available at www.wheatgenome.info

Designing 7DS specific SNP markers

- Syntenic regions in genomes of barley, rice, *Brachypodium* and sorghum aligned in GenomeZipper corresponding to our region of interest were delimited
- Barley ESTs from HarvEST database with a known position on barley genetic map were blasted against the wheat 7AS, 7BS and 7DS assemblies
- Contigs containing sequences homologous to the barley ESTs were identified

GenomeZipper

marker	cM	brachypodium hit	rice hit	sorghum hit	shotgun reads	corresponding harvEST35
-	-	Bradi3g39340.1	Os08g0499300	Sb07g028420.1	FULMQYZ01EL	-
-	-	-	-	Sb07g028440.1	FULMQYZ01CS	-
-	-	Bradi3g39350.1	Os08g0499200	Sb07g028460.1	FULMQYZ01AC	38029:7349
-	-	Bradi3g39360.1	Os08g0499100	Sb07g028470.1	FULMQYZ02GS	31990:42871
-	-	Bradi3g39400.1	Os08g0498400	Sb07g028490.1	FULMQYZ01CS	-
-	-	-	-	Sb07g028520.1	FULMQYZ02FE	14101
-	-	Bradi3g39420.1	Os08g0498100	Sb07g028530.1	FULMQYZ02FE	14101
-	-	Bradi3g39440.1	Os08g0497900	-	FULMQYZ02HH	11935
-	-	-	-	Sb07g028550.1	FULMQYZ01BD	19275
-	-	Bradi3g39470.1	Os08g0497300	Sb07g028560.1	FULMQYZ01DH	17996:4318
1_0983	75	Bradi3g39480.1	Os08g0496900	Sb07g028570.1	FULMQYZ01AL	4037
-	-	Bradi3g39490.1	Os08g0496800	Sb07g028580.1	FULMQYZ01EE	1376:27758
-	-	Bradi3g39500.1	Os08g0500000	Sb07g028380.1	FULMQYZ01AZ	16247
-	-	-	-	Sb07g028350.1	FUYK5KB01AM	10225
-	-	Bradi3g39510.1	Os08g0500100	Sb07g028340.1	FULMQYZ02FK	3339:40799:18447
-	-	Bradi3g39520.1	Os08g0500200	Sb07g028330.1	FULMQYZ02HA	12579:21535
-	-	Bradi3g39540.1	Os08g0500300	Sb07g028300.1	FULMQYZ01CI	10513
-	-	Bradi3g39550.1	Os08g0500500	Sb07g028290.1	FULMQYZ01EE	21376:38836
-	-	Bradi3g39590.1	-	-	FULMQYZ01D7	14120
-	-	-	-	Sb07g028230.1	FULMQYZ01E2	-
-	-	Bradi3g39600.1	Os08g0500800	Sb07g028250.1	FULMQYZ01AR	14162:14151
-	-	Bradi3g39610.1	Os08g0500600	Sb07g028280.1	FULMQYZ01CF	-
2_1276	75	Bradi3g39620.1	-	Sb07g028270.1	FULMQYZ01D	14117:14120
-	-	Bradi3g39630.1	Os08g0500700	-	FULMQYZ01D7	14120
2_1167	75	Bradi3g39640.2	-	-	FULMQYZ02HI	18557

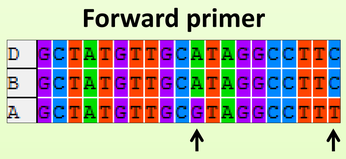


GenomeZipper was created by combining *Brachypodium*, rice and sorghum gene sequences, 454 reads of particular barley chromosomes and barley ESTs.

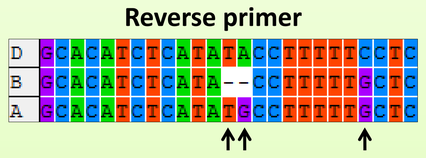
Syntenic regions in rice, *Brachypodium* and sorghum identified by GenomeZipper

- 7AS, 7BS and 7DS sequences were aligned and SNPs and/or INDELs were identified

Forward primer

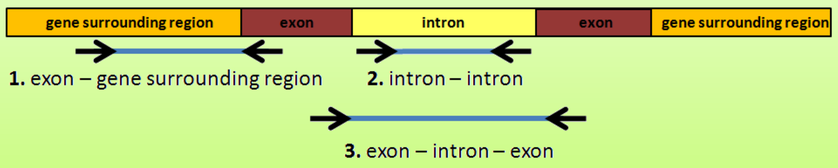


Reverse primer

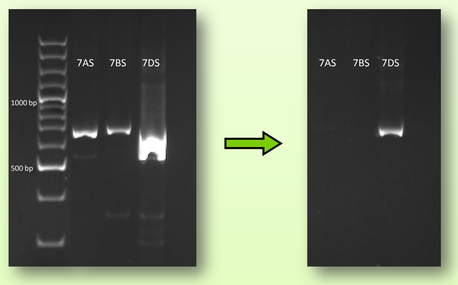


- 7DS specific primers were designed

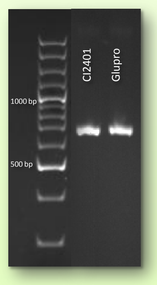
3 types of primer pairs:



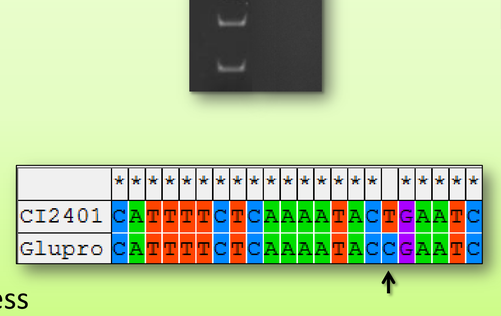
- After optimizing PCR conditions, 7DS specific product was obtained

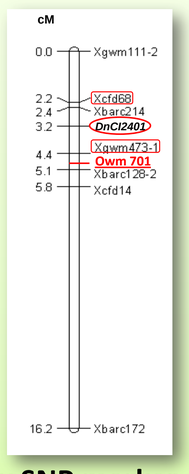


- 7DS specific sequences were amplified from DNA of parents of mapping populations



- PCR products were sequenced and SNPs between parents of mapping populations were identified.
- Mapping of the SNPs is in progress





New SNP marker Owm701 was placed on genetic map in the *DnCI2401* region

Results

- In total, 9 SNPs in both mapping populations were identified
  - 5 in gene surrounding regions
  - 3 in introns
  - 1 in exon

Mapping population	Sequences compared	Number of SNPs	Frequency of SNPs
CI2401 x Glupro	11,600 bp	2	1 SNP / 5,800 bp
ITMI	5,300 bp	7	1 SNP / 750 bp

- New SNP marker **Owm701** was placed on genetic map close to the *DnCI2401* gene, confirming colinearity between wheat and barley in the *DnCI2401* region.

Conclusions

- The presented approach employing sequence information from individual wheat chromosome arms helps reduce problems associated with polyploidy and enables targeted development of markers from the region of interest
- Most of the SNPs were found in gene surrounding regions, which will be a target for the future SNP identification
- The present mapping population (CI2401 x Glupro) shows very low level of polymorphism → development of a new mapping population including synthetic wheat will be essential

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