Colinearity-based marker mining for high density mapping of the wheat Powdery mildew resistance locus QPm.tut-4A

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Introduction

Bread wheat (*Triticum aestivum* L.) is one of the world's most important crops providing staple food for 35% of the world's population and 20% calories consumed. Maintaining stable yield of wheat is annually challenged by attack of large variety of diseases and pests. Breeding for host resistance is the most efficient, economical, and environmentally safe approach to protect wheat from pathogens and secure stable yield. Development of new markers, construction of high density maps, and positional cloning of resistance genes are essential conditions of this approach.

Wheat powdery mildew disease is caused by fungus *Blumeria graminis* DC. f. sp. *tritici*. It is an obligate, biotrophic parasite that is potentially destructive under humid rain fed and cool temperature conditions and in areas under irrigation. Grain yield losses are related to reductions in grain size and number per unit area and could exceed 40%. Recently, segments of the tetraploid genome of *Triticum militinae* were introgressed to common wheat cultivar 'Tähti'. The hybrids are characterized by improved seedling (SPR) and adult (APR) plant resistance to powdery mildew.

Two main QTLs located on two different chromosomes (4A and 5A) were found to control the quantitative resistance to powdery mildew in the mapping population consisted of 134 F2 plants and 140 double haploids (DH) derived from F3 families. The mapping population was derived from the cross *T. aestivum* 'Tähti' with the hybrid plant 8/1 (APR, *T. militinae* x 'Tähti'). The main QTL responsible for APR was localized on the long arm of chromosome 4A, in region flanked by markers *gwm160* and *wmc232* on a *T. militinae* translocation and explained 54% of the phenotypic variance (Jacobson *et al.,* 2006). The APR resistance gene was designated as *QPm.tut-4A*. No linkage to any previously mapped powdery mildew resistance gene has been detected. For successful cloning of the gene saturation of genetic map of the region is essential.

RESULTS

Saturation of the QPm.tut-4A gene region with new markers

In effort to clone the *QPm.tut-4A* gene we saturated the gene region with SSR markers from colinear wheat maps. Twenty six new markers were tested (Table 1) and only the markers *barc70, barc78*, *gwm832,* and *gwm855* were polymorphic in the region. The all four markers were mapped completely linked to the marker *gwm160* using 1111 gametes. No more SSR marker for the region is available at present and identification of additional marker sources is necessary for successful cloning the *QPm.tut-4A* gene.

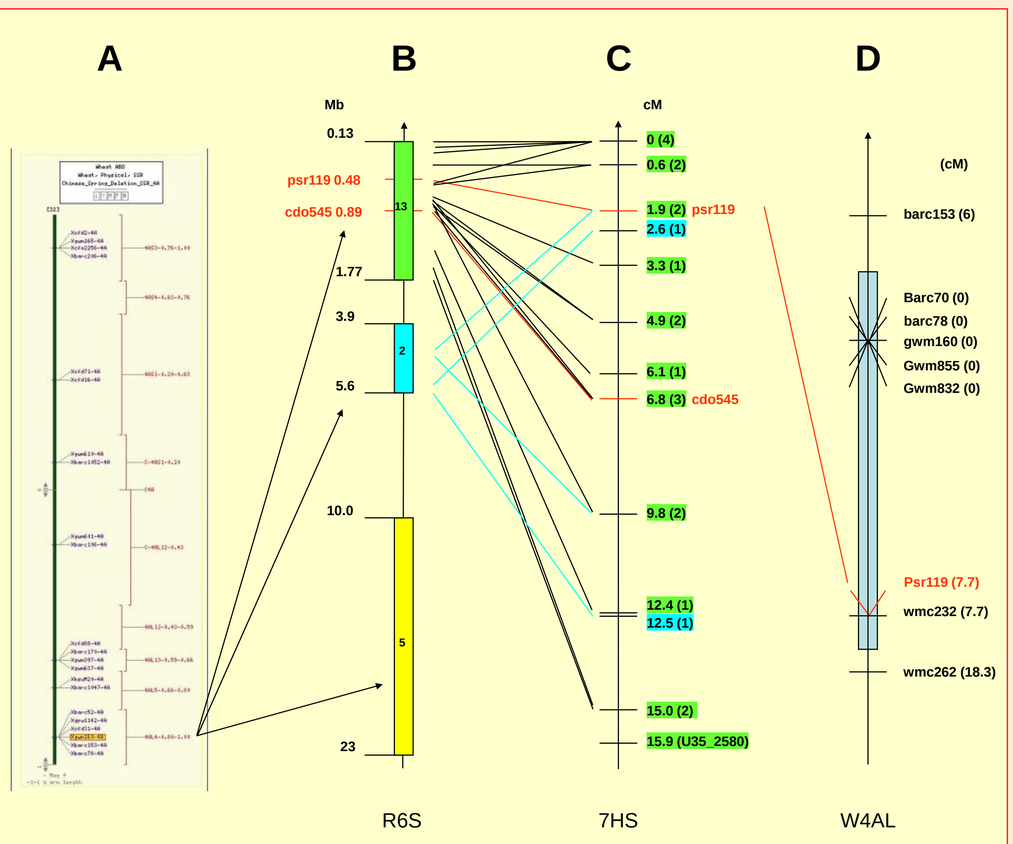

Figure 1. Colinearity of between wheat, rice, and barley at *QPm.tut-4A* gene region.

A) SSR deletion map of the wheat 4A chromosome. Note the gwm160 mapped to the most distal bin of W4AL. **B)** Segment of short arm of rice chromosome 6 (R6S) with mapped single copy ESTs from the last W4AL bin. Note that the color of rectangles corresponds with EST groups at Table 1 and numbers inside of the rectangles indicate number of mapped colinear wheat ESTs. **C)** Detailed EST map of distal part of barley chromosome arm 7HS (http://harvest.ucr.edu/) colinear to the *QPm.tut-4A* gene region. Color label correspond to EST grouping in Table 2. **D)** Genetic map of the *QPm.tut-4A* gene region using 871 gametes of our mapping population. The black rectangle represents 4A translocation from *T. militinae.* Numbers in brackets indicate genetic distance of the marker *gwm160* position. Note: The red labels indicate position of markers used as colinear sequence anchors from different wheat maps for the *QPm.tut-4A* gene region.

Table 1.Position of wheat ESTs from the last bin of wheat chromosome arm 4AL (W4AL) on colinear rice chromosome 6 (R6). Different colors indicate groups of the ESTs mapping together on the R6 (Figure 2B). Note that the majority of the ESTs maps to the very end of the short arm of rice chromosome 6 (Figure 2B).

EST from 4AL4-0.80-1.00	Rice position
gb|BE586111.1|BE586111 Est#3SP6_C05_c5_034 KSU wheat Fusarium gr 66 2e-008	23 M
gb|BE444558.1|BE444558 WHE1126_A07_A14ZS Wheat etiolated seedlin 111 4e-022	17 M
gb|BF201403.1|BF201403 WHE1770_C04_F08ZS Wheat pre-anthesis spik 60 1e-006	10 M
gb|BE444404.1|BE444404 WHE1118_G05_M10ZS Wheat etiolated seedlin 76 2e-011	10 M
gb|BE426800.1|BE426800 WHE0332_C07_F14ZS Wheat unstressed seedli 54 7e-005	10 M
gb|BE498428.1|BE498428 WHE0967_H04_O07ZS Wheat pre-anthesis spik 121 4e-025	4,8 M
gb|BE586056.1|BE586056 Est#3pT7_C07_c7_050 KSU wheat Fusarium gr 68 5e-009	3,9 M
gb|BE606874.1|BE606874 WHE0902_B02_C04ZS Wheat 5-15 DAP spike cD 188 2e-045	1.77 M
gb|BE490191.1|BE490191 WHE0366_D10_H20ZS Wheat cold-stressed see 262 2e-067	1.73 M
gb|BG607226.1|BG607226 WHE2472_H11_O22ZS Triticum monococcum ear 92 3e-016	1,6 M
gb|BG604519.1|BG604519 WHE0834_F06_K12ZS Wheat vernalized crown 297 3e-078	1,6 M
gb|BE498338.1|BE498338 WHE0952_G06_M12ZS Wheat pre-anthesis spik 88 5e-015	0,9 M
gb|BE517802.1|BE517802 WHE0803_F03_L05ZS Wheat vernalized crown 174 3e-041	0.86 M
gb|BE443120.1|BE443120 WHE1113_D10_G19ZS Wheat etiolated seedlin 72 3e-010	0,7 M
gb|BF429350.1|BF429350 WHE1804_D12_H24ZS Secale cereale anther c 339 8e-091	0,6 M
gb|BE499049.1|BE499049 WHE0960_H01_O02ZS Wheat pre-anthesis spik 456 e-126	0,6 M
gb|BE443444.1|BE443444 WHE1104_B03_D06ZS Wheat etiolated seedlin 216 8e-054	0,6 M
gb|BE638019.1|BE638019 WHE0995-0998_K05_K05ZS Wheat pre-anthesis. 274 5e-071	0.55 M
gi|32555493|gb|CD871677.1| Ogihara unpublished cDNA library 210 2e-52	0.55 M
gb|BF200736.1|BF200736 WHE0821-0824_M13_M13ZS Wheat vernalized c 58 5e-006	0,38 M

RESULTS

Exploring a colinearity between wheat and rice, barley and Brachypodium at QPm.tut-4A gene region

Wheat vs. rice: To generate new markers for the *QPm.tut-4A* gene region we employed colinearity between wheat, rice, barley, and Brachypodium in gene order and available physical map of wheat. The marker *gwm160* was previously mapped in the most distal bin 4AL4-0.80-1.00 on the deletion map of the wheat chromosome arm W4AL. To the bin which represents about 20% of the 4AL chromosomal arm, 91 single copy ESTs were mapped. All the ESTs were mapped to rice genome to test if they allow us to identify the colinear rice genome segment. From the 91 ESTs 48 had orthologous genes in rice chromosomes R1-R6. On the chromosome R6, 20 of the 48 ESTs were mapped (Table 1, Figure 1A, B) where the majority of the ESTs (13) were mapped to the relatively narrow region of the R6S from 0 to 1.77 Mb (starting from telomere) and is considered as the major colinear region to the wheat bin 4AL4-0.80-1.00. Remaining seven ESTs were scattered in two smaller groups in the R6 regions from 3.9 to 23 Mb (Figure 1B). To verify the assumption that the 1.6 Mb R6S region is colinear with the *QPm.tut-4A* gene region, we searched in available wheat maps using Graingene CMap tool (http://wheat.pw.usda.gov/cmap/) for markers consistently linked to the markers *gwm160* and *wmc232* as tightly as possible and derived from publicly available sequence. The markers *psr119* and *cdo545* fulfilled the conditions. The sequence of the marker *cdo545* is on 88% identical to rice Serine/threonine-protein kinase MAK gene NP_001056617.1 and the *psr119* is on 89% identical to rice CYPOR like ferredoxin reductases gene NP_001056570.1. The *cdo545* is localized on R6 at position 0.89 Mb and *psr119* at position 0.48 Mb. The *psr119* was mapped linked to the marker *wmc232* in our mapping population. All these results are supporting the assumption that the R6S end of the chromosome is colinear with the W4AL end of chromosome in the *QPm.tut-4A* gene region. The 1.6 Mb of the R6S region contains 173 genes suitable as marker source for saturation of high density map of the *QPm.tut-4A* gene region. The R6 region showed colinearity with the wheat chromosomes W7AS, W7DS and barley chromosome7HS and not with the group of chromosomes 4. These findings are suggesting that the *QPm.tut-4A* gene region could be on ancestral segment of chromosome 7BS translocated to the chromosomal arm 4AL.

Wheat and Rice vs. Barley: In attempt to explore barley mapped ESTs as marker source for the *QPm.tut-4A* gene region, we focused on colinearity between barley chromosome 7H and rice in the R6S 0-16 Mb region using HarvEST software (http://harvest.ucr.edu/). Overall colinearity between R6 and 7H is well preserved and we delimited region colinear to the rice candidate region by mapping the most proximal gene from the rice region the MutT/nudix protein-like - Oryza sativa (japonica cultivar-group) from position 1.6 Mb to the barley map (Figure 1C). The rice protein is similar to the barley UniGene U35_3778 (e-125). The unigene U35_3778 was mapped at 15.0 cM measured from the end of the 7HS chromosomal arm. Based on this data, we selected the region 0-16 cM of the barley map as potential source for markers in the *QPm.tut-4A* gene region. 48 barley ESTs, which will be explored as potential source of markers for the *QPm.tut-4A* gene region, were mapped to this region. From these 48 ESTs 4 have homologous ESTs mapped in the last bin of W4AL but not in rice and 32 have homologous genes in rice genome (Table 2). Four of the 32 ESTs were mapped distal to the R6 region of interest (Figure 1C, blue lines) and nine of the ESTs were mapped to the different rice chromosomes (Table 2) and those 13 ESTs can be excluded from the marker development effort. Majority of the ESTs (19) were mapped to the end of R6S within 0-1.6Mb (Figure1B, C, Table2).

Rice vs. Bbrachypodium: The genome of Brachypodium distachyon (http://www.brachypodium.org) was compared with the most distal R6S region to gain new potential markers and verify the colinearity between these two species. The *psr119* marker was mapped in the Brachypodium genome on chromosome 1 at position 50.4 Mb. Colinearity in approximately 100 kb regions spanning the *psr119* locus in both genomes was investigated. The regions were annotated and compared using Artemis Comparison Tool (ATC, http://www.sanger.ac.uk/Software/ACT/, Figure 2). Colinearity in these two regions is well preserved in gene number (Brachypodium 18 and rice 19) and order except that whole regions are in +/- orientation, the region 0.44-0.45 Mb of R6 which contains four genes was inverted compared to colinear Brachypodium region and their flanking regions (Figure 2, the blue lines), Brachypodium orthologous sequence of subtilase-like gene BAA90633 was localized on chromosome 1 at position 20.7 Mb, and hypothetical protein OsI_21301 at position 0.38 Mb does not have Brachypodium orthologous gene suggesting insertion/deletion/translocation for these regions.

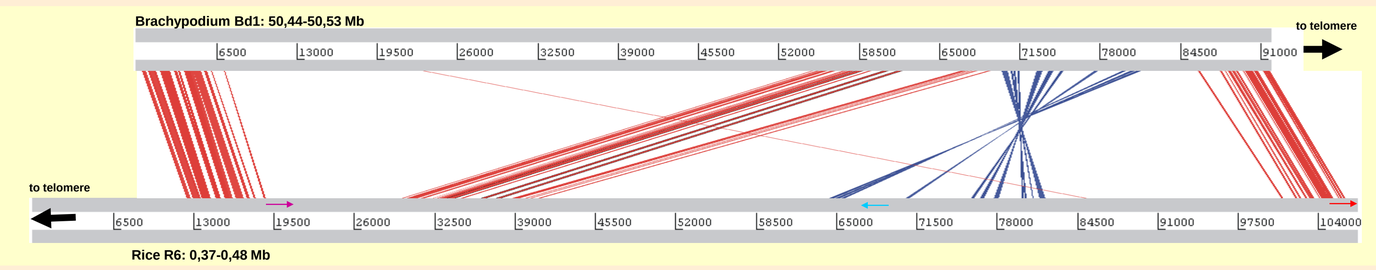

Figure 2. Comparison of approximately 100kb of Rice and Brachypodium regions from *psr119* loci.

For comparison of the regions Artemis Comparison Tool (ATC, http://www.sanger.ac.uk/Software/ACT/) was used. The rice gene corresponding to psr119 marker is indicated by red arrow. The regions have well preserved gene order and orientations (the red lines) except inversion containing four genes (blue lines), a non-homologous rice gene (blue arrow), and a gene with homology to a different region of Barachypodium chromosome 1 (the purple arrow). Also the DNAs are in +/- orientation indicated by black arrows pointing toward the telomeres.

Table 2. Distribution of mapped barley ESTs (unigene probes) from 7HS region colinear to *QPm.tut-4A* gene region. The 7HS region 0-16 cM was identified as colinear to wheat *QPm.tut-4A* gene region using HarvEST softvare (http://harvest.ucr.edu/). All 48 identified probes were mapped to rice genome. Bolded lines indicate probes similar to wheat ESTs mapped in colinear wheat chromosomal locations. Different colors indicate colinear location with wheat ESTs mapped to R6S (Figure 2B, C). ESTs from the Group 4 does not have rice homologous genes but have wheat homologous ESTs mapped to the colinear wheat positions.

	Barley probe	Barley map position	Position in rice			Wheat Bin
			Copy 1	Copy 2	Copy 3	
Group 1	U35_551	12.5	R6 5.6 Mb			NO
	U35_19120	2.6	R6 5.55 Mb			NO
	U35_14480	9.8	R6 4.8 Mb	R3 16.1 Mb.	R7 25.4 Mb.	7AS5-0.59-0.89 7BS1-0.27-1.00
	U35_15004	1.9	R6 4.8 Mb.	R2 1.8 Mb.	R12 1.01 Mb	C-2AS5-0.78, 6AS5-0.65-1.00, 6DS6-0.99-1.00
Group 2	**U35_479**	**0**	**R6 0.5 Mb**	**R2 0.29 Mb**	**R7 12.7 Mb**	**7AS1-0.89-1.00**
	U35_15280	0	R6 0.23 Mb			NO
	U35_56	0	R6 0.15 Mb			NO
	U35_20037	0	R6 0.13 Mb			NO
	U35_4425	6.1	R6 0.86 Mb			NO
	U35_2649	3.3	R6 0.74 Mb			NO
	U35_814	12.4	R6 1.35 Mb	R11 10.7 Mb		NO
	U35_1490	6.8	R6 0.9 Mb	R7 3.32 Mb		NO
	U35_1176	6.8	R6 0.9 Mb			NO
	U35_1550	6.8	R6 0.7 Mb			NO
	U35_969	9.8	R6 0.85 and 0.83 Mb	R2 23.9 Mb	R11 3.0 Mb	NO
	U35_17833	1.9	R6 0.44 Mb.	R3 1.4 Mb		4BL5-0.86-1.00
	U35_17190	4.9	R6 0.8 Mb			NO
	U35_802	**4.9**	**R6 0.6 Mb**	**R11 16.5 Mb**		**4AL4-0.80-1.00 7AS1-0.89-1.00**
	U35_481	0.6	R6 0.5 Mb	R2 0.6 Mb		1BS.sat18-0.50-1.00
	U35_19640	**0.6**	**R6 0.4 Mb.**	**R2 0.28 Mb**		**4AL4-0.80-1.00**
	U35_3778	15.0	R6 1.6 Mb			NO
	U35_3726	15.0	R6 1.5 Mb	R9 20.6 Mb		NO
	U35_17115	4.1 and 4.9	R6 0.7 Mb			NO
Group 3	U35_2580	15.9	R4 35.5 Mb			
	U35_1998	4.9	R4 19.5 Mb			
	U35_15653	0		R2 0.35 Mb.	R4 26.4 Mb	
	U35_5047	1.9	R12 15.4 Mb			NO
	U35_15984	0.6	R12 14 Mb.	R3 16.3 Mb		NO
	U35_1995	**0.6**	**R12 13.9 Mb**	**R3 16.5 Mb and 11.3 Mb**		4AL13-0.59-0.66 **7AS1-0.89-1.00**
	U35_47650	**0**	**R12 13.9 Mb.**	**R3 16.26 Mb**		4AL13-0.59-0.66 **7AS1-0.89-1.00**
	U35_5016	0	R11 13.5 Mb			
	U35_4432	1.9	R10 10.7 Mb			
Group 4	**U35_4432**	**1,9**				**4AL4-0.80-1.00**
	U35_3681	**3,3**				**4AL4-0.80-1.00**
	U35_14422	**5,5**				**4AL4-0.80-1.00**
	U35_20056	**15,0**				**4AL4-0.80-1.00**

CONCLUSIONS

- **Genetic map of the *QPm.tut-4A* gene region was saturated with all available SSR markers.**
- **Colinear regions of *QPm.tut-4A* locus in wheat, rice, barley, and Brachypodiium were identified and explored for genes as new marker sources.**
- **The colinearity at the identified regions was well preserved and the most probable *QPm.tut-4A* colinear regions in rice and Brachypodium genome sequences were identified.**
- **Anchoring the *QPm.tut-4A* locus to wheat deletion map and rice chromosome 6 yielded 173 genes as potential marker source.**
- **Identification of barley colinear region yielded 16 ESTs mapping to the colinear barley region but not to rice. Four of those have homologous wheat ESTs mapped to the colinear bin.**
- **Identification of Brachypodium region colinear to the *QPm.tut-4A* region confirmed strong preservation of gene content in the region but did not yield more genes as marker sources.**
- **Employing a colinearity study in the *QPm.tut-4A* gene region between wheat, rice, barley, and Brachypodium facilitated identification of 189 genes as a sources of new marker for the region.**

REFERENCES

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