GENETIC MAPPING AND QTLs ANALYSIS OF POLLEN STERILITY IN TRITICALE WITH TRITICUM TIMOPHEEVII STERILIZING CYTOPLASM

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INTRODUCTION

Cytoplasmic-nuclear male sterility (CMS) is a maternally inherited inability of plants to produce functional pollen. CMS is due to the dysfunction between nuclear and the mitochondria genomes and could be compensated by nuclear pollen fertility genes (Rf). The function of such genes is usually purely known; however, they are of value for many breeding applications as they allow for the control over parental materials and may result in increased yield due to heterosis. 10-20 % relative midparent grain yield was documented in such systems, i.e., in rye with CMS Pampa. There is a great interest in the development of such systems in the other crops including wheat and further progress in triticale.

As the genetic background of pollen fertility is mostly hardly known, the relevant studies in crops are needed. Genetic mapping of the QTLs responsible for the expression of Rf genes or association mapping followed by the identification of molecular markers tightly linked to or associated with such genes may be the method of choice here. The identified markers could be used in marker-assisted programs having an impact on new hybrid variety development

MATERIAL AND METHODS

Biparental recombinant inbred line mapping population encompassing 184 lines was evaluated (RIL6: ms HT 112(15)-2-1 x Borwo). The population was evaluated via crossing a single plant of a male sterile line (ms HT 112(15)-2-1) to a single plant of Borvo cultivar followed by six cycles of selfing of the F2 progeny of the cross.

Pollen fertility restoration was evaluated based on the phenotype of the BC1F6: ms HT112(15)-2-1 cms Tt x ms HT112(15)-2-1 x Borwo. The number of seeds in the BC1F6 spikes was counted.

The RIL6: ms HT 112(15)-2-1 x Borwo mapping population was genotyped with DArTseq and silicoDArT markers and the segregation were used for the construction of genetic map using Ultra Dense MultiPoint software.

Composite interval mapping (CIM) was used to identify QTLs and markers linked to the trait. CIM was performed in WinQTL Cartographer. In CIM the following parameters ‚Walk Speed' – 1, Window Size' – 1, Markers' – 15 were used. The permutation test (1000 permutations) was applied. Association mapping was run in Gapit (R-cran scan).

RESULT

Composite mapping

Composite interval mapping allowed the identification of three QTLs conferring pollen fertility restoration QTLs with LOD function maximum above the cut-off value evaluated by the permutation test.

The QTLs were mapped to the linkage groups LG16(3R), LG19(6B) and LG25(4R) (Figure 1). The detailed data concerning QTLs are given in Table 1.

Figure 1. Composite interval mapping based on the genetic map developed on RIL6: ms HT112(15)-2-1 x Borwo and pollen fertility restoration data (seeds per spike) evaluated using the BC1F6: ms HT112(15)-2-1 cms Tt x ms HT112(15)-2-1 x Borwo population.

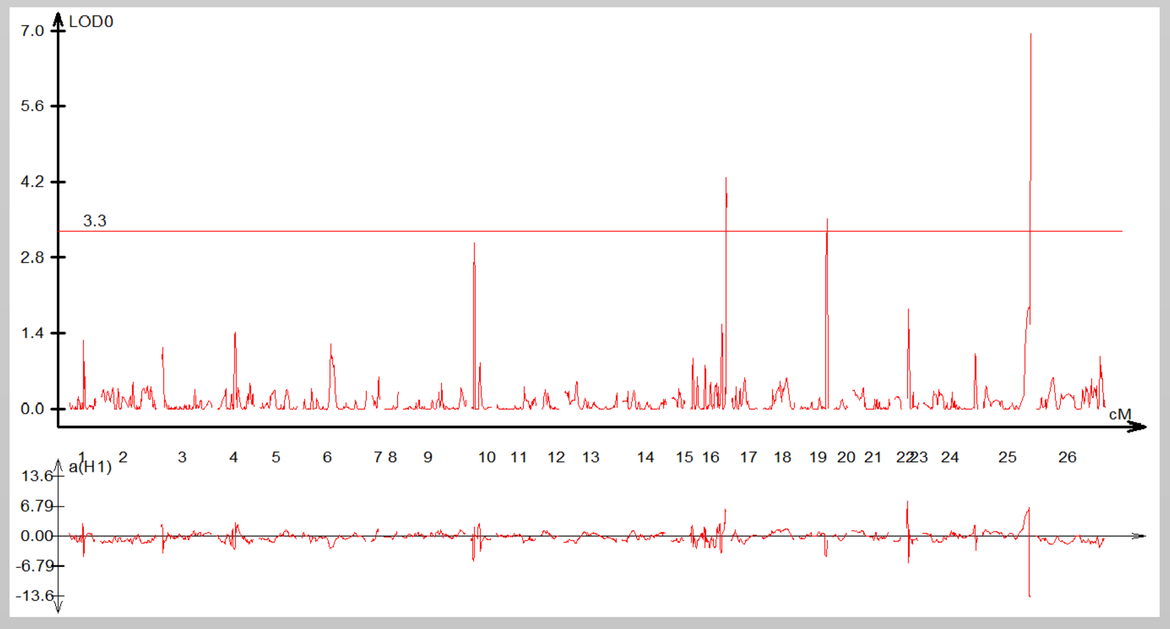


Table 1. Arrangement of markers data mapped within QTL regions.

QTL	QTL 1	QTL 2	QTL 3
QTL assignment	LG16 (3R)	LG19 (6B)	LG25 (4R)
Marker within QTL maximum	SD_3622212	DS_8512159_C>G	SD_16352942
QTL maximum	4,28	3,53	7,1
Marker position (cM)	78,3	59,4	104,4
Markery within QTL	SD_4214525	SD_4203022	SD_4561254
	SD_3622212	DS_8512159_C>G	SD_163242
	SD_10495200	DS_3047671_A>G	-
QTL range (cM)	78,1-79,84	56,5-61,4	102,7-104,85
Additivity value	6.3142	-4.6336	-
R2	0.0555	0.0477	-
TR2	0.5167	0.5184	-
S	3.5996	1.9806	-
RecL	0	0.0004	-
RecR	0.0062	0.0179	-

RESULT

Genetic map

Genotyping allowed for the identification of as many as 19967 DArTseq nad 69231 silicoDArT markers. Monomorphic markers and those with over 70% missings were removed from the analysis. For genetic mapping, a set of 25066 was used (Table 2).

Genetic mapping enabled the identification of 26 linkage groups (LG). There were 3499 markers on the map (1093 skeleton and 2356 redundant markers). In total, all LGs covered 1093 cM and the markers were distributed every 2.00 cM.

Based on known chromosomal location of DArTseq and silicoDArT markers in wheat, 10 LGs were assigned to genomes A and 9 to B. Using genome collinearity data, the remaining 7 LGs were assigned to the R genome.

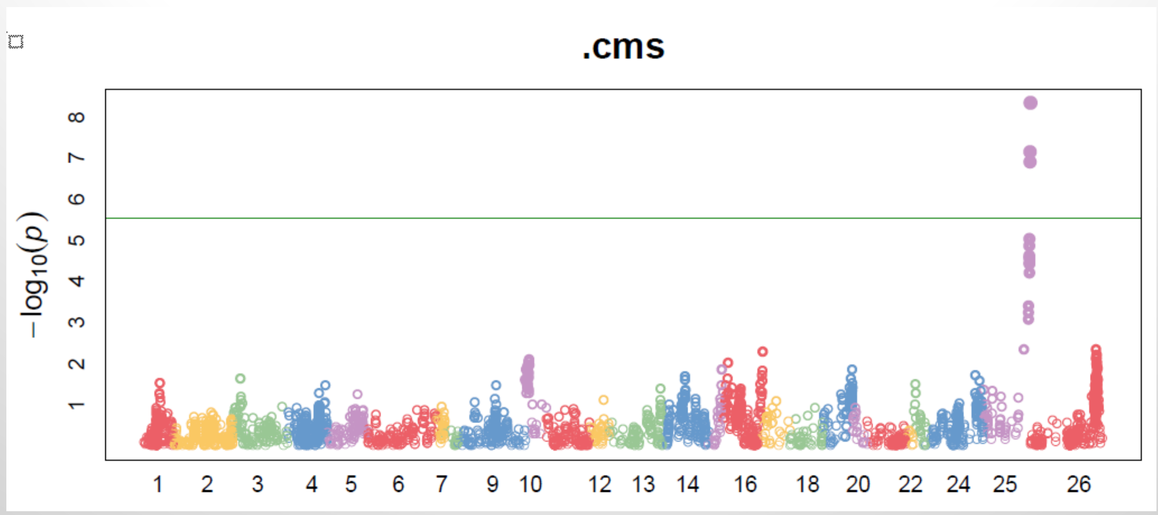
Table 2. The arrangement of data concerning genetic map of triticale constructed on the RIL6: ms HT 112(15)-2-1 x Borwo mapping population encompassing 184 individual RILs.

LG	Chromosome	LG lenght (cM)	Skeleton markers	Redundant markers	Total numer of markers	Marker density (cM)
LG1	1R	58,74	52	283	2479	1,13
LG2	2B	121,85	90	269	2027	1,35
LG3	5B	111,3	69	64	972	1,61
LG4	5R	80,96	66	272	2294	1,23
LG5	3A	83,57	44	55	413	1,90
LG6	2A	140,61	66	80	598	2,13
LG7	7B	17,04	16	18	576	1,07
LG8	2R	30,65	11	18	457	2,79
LG9	3B	137,61	69	113	1310	1,99
LG10	1A	44,56	17	24	177	2,62
LG11	7A	87,42	58	85	976	1,51
LG12	1A	39,4	24	16	217	1,64
LG13	5A	115,9	41	73	546	2,83
LG14	6A	96,66	52	106	672	1,86
LG15	3A	29,48	17	17	284	1,73
LG16	3R	79,84	70	139	2259	1,14
LG17	4B	57,34	22	12	100	2,61
LG18	4A	69,91	28	32	252	2,50
LG19	6B	62,66	25	41	823	2,51
LG20	5B	31,08	12	10	80	2,59
LG21	7R	80,43	48	60	743	1,68
LG22	5A	16,45	7	3	46	2,35
LG23	4B	25,35	15	13	80	1,69
LG24	1B	119,64	63	125	1036	1,90
LG25	4R	104,85	27	38	2069	3,88
LG26	6R	153,59	84	390	3580	1,83
ŁĄCZNIE		1996,72	1093	2356	25066	-
ŚREDNIA		76,8	42,04	90,62	964,08	2
MINIMUM		16,45	7	3	46	1,07
MAKSIMUM		153,59	90	390	3580	3,88

Association mapping

Association mapping confirmed the presence of a single QTL on the 4R chromosome. The location of the associated markers falls in the QTL region evaluated via CIM. No other QTLs were confirmed using AM (Figure 2).

Figure 2. Manhattan plot illustrating the chromosomal location of the markers associated with pollen fertility genes using genetic map (Figure 1). The X-axis reflects successive LGs, while the Y-one, the probability value (in the logarithmic scale) of the marker-trait association.



CONCLUSION

The saturated genetic map of triticale designed on the RIL6: ms HT112(15)-2-1 x Borwo mapping population dedicated for the given study was evaluated.

The linkage groups were assigned to the appropriate chromosomes of the species based on known location of the DArTseq and silicoDArT markers on wheat genetic maps and using synteny.

Composite interval mapping confirms the multigenic nature of the pollen fertility restoration trait in triticale with the CMS Tt system and allowed for the identification of three QTLs conferring the trait.

Out of the three QTLs, the one on the chromosome 4RL proved to be the most significant one.

The markers tightly linked to the QTLs were identified for further MAS purposes.

Association mapping also indicated that the QTL on the 4R chromosome is the most important one, at least in the case of the given mapping population and allowed the identification of some extra markers associated with the trait of interests.