Identification of pollen fertility restoration markers in rye with CMS Pampa

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INTRODUCTION

The cytoplasmic male sterility (CMS) phenomenon in plants is based on incompatibility of nuclear and cytoplasmic genomes and results in the lack of production of functional pollen. Its implementation into breeding systems of cereals led to the development of hybrids of commercial importance. A good example is an exploitation of heterosis in hybrid rye with CMS-Pampa (Geiger, Schnell 1970). The evaluation of new hybrids in rye requires efficient parental lines that can restore pollen fertility. Pollen fertility is expressed by numerous genes (Miedaner, Glass et al. 2000), and those located on the chromosome 4R (and 1R) explain most of the phenotypic variance of the trait. For marker-based breeding purposes, markers towards that genes are required.

MATERIAL AND METHODS

The plant material consisted of RIL4 individuals that originated from a biparental cross (maintainer (N) x restorer line with CMS Pampa). RIL4 and parental lines were genotyped with DArTseq and DArT markers. Pollen fertility restoration of the RILs was evaluated via phenotyping BC_0F_3 materials derived via backcrossing of the CMS Pampa maternal line and RIL4 lines using visual bonitation scale (Geiger, Morgenstern 1975). A genetic map was constructed under MultiPoint software (http://www.multiqtl.com). The map was visualized in MapChart (Voorrips 2002). Association mapping was performed in TASSEL (Bradbury et al. 2007). Composite interval mapping was conducted in Windows QTL Cartographer (Wang et al. 2012).

RESULTS

1. The genetic map consisted of seven linkage groups corresponding to 7 rye chromosomes covering 962 cM (Figure 1).
2. As many as 528 DArTseq and 43 DArT markers were mapped with a few gaps (the largest spanned over 27 cM).
3. Composite interval mapping allowed for the identification of a QTL located on the chromosome 1R (covering over 30 cM) with LOD function maximum equal to 30.8. The closest to the QTL marker was 1.9 cM from its maximum (Figure 2).
4. Association mapping allowed the identification of 292 markers that passed Bonferroni test with the R2 ranging from 0.12 to 0.56 (Table 1). Some of the associated markers mapped to the 1R chromosome and felt within the pollen fertility restoration QTL.
5. Based on segregation data some of the DArTseqs formed 14 redundant groups located within QTL. One of the groups, the closest (~2 cM) to the QTL maximum, consisted of 1 skeleton and two redundant markers with R2 equal to 0.56.

Figure 1. The genetic map constructed based on RIL4 and DArTseq/DArT markers. Associated markers are marked in red. The most associated marker is marked in green. The marker closest to the QTL maximum is given in dark blue. The QTL region is indicated in light blue.

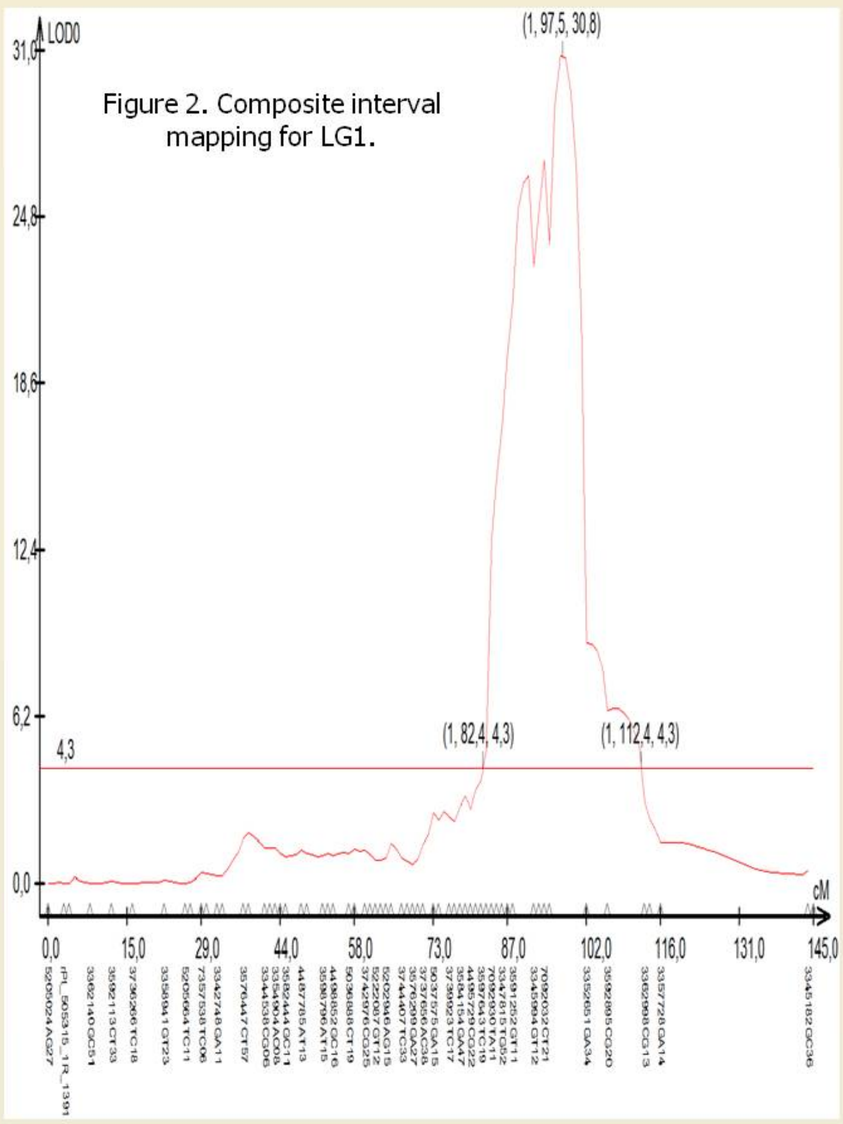

Figure 2. Composite interval mapping for LG1.

Table 1. The arrangement of the data concerning pollen markers associated wth pollen fertility trait

Marker	SNP	SNP Position	Allele Sequence	Marker R^2
4484564AC22	A>C	22	TGCAGCGAGTGAAATGAAATTCAAGCGAACCGAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCG	0,5597
3596256GC41	G>C	41	TGCAGGTAGTCCAGCTTCGCCTTCTCCAGATCCGCCATCACGCGATCCGCCTTGTATATCCGAGATCGG	0,5571
3895288CA53	C>A	53	TGCAGCTGCTGGCCCAGCTGCTCGACGAGGTGACCGCGCTCTCGCCGTCCATCCCTTCCCTGAGCAGAA	0,5570
5502538AG08	A>G	8	TGCAGCGCAGCGAGCTTGGCGAGGGACCCCGAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGA	0,5568
3363491AG19	A>G	19	TGCAGCCTTCCTGGAACATAGTGACGGCCTTTTCTCCGTCGGACTCCCCGCCGAGATCGGAAGAGCGGT	0,5567
7092032CT21	C>T	21	TGCAGGAGGAGTTCGGCGGGGCGGAGGGCGCCGAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACC	0,5567
3593524GC21	G>C	21	TGCAGGGATGGAGAAGCAGGAGAAGGGCGGCGAGATGGGCAGGCTGGGCGGCTGCGCCGACTTGAACTT	0,5567
3354171GA24	G>A	24	TGCAGGACGCGGGTCGCCACCGAGGTTGTCAGAGTTGGCTTGCAGTAGCTTCCTCCTTGATCTCTGCTG	0,5565
4486686AG09	A>G	9	TGCAGGATGATGACGGCCCTGAGCCGAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCTCG	0,5289
3897679CG45	C>G	45	TGCAGCCACGGCCGTGTATCACGGCCTGGCCGTTGGTCGATGTGCCACACTCCGCGTCAGCCTGCCCTG	0,5263
3902666GA51	G>A	51	TGCAGCGTTTGTGCAGCCGACGCACTCTAAGTGCTCGATGAAATGCCCATTGGAGAGATAGGAGGAGGG	0,5230
4104069AG51	A>G	51	TGCAGGCCACGGAGGGCGTGGCACGGGTGAGGGCGGCAAGGAATCCGTTCAAGGAGCGCTCAGGGACCG	0,5229
5217232TA51	T>A	51	TGCAGTAGGCTACTTGGGTCGGCCAACCAGACGCTCGCTATAGCAGAGGGGTGTTTCTGTCTCGCGTCA	0,5188
3738683CT11	C>T	11	TGCAGCCTCCCCGAGCCGACGCCCAGCCCCGCCGACATCGCCGCCATCAGCCGCTCGCACAGCGACGCC	0,5153
5040487AG07	A>G	7	TGCAGAGACTGCCGGCTCGTGTCCTCGAGACGCTTGGCCAGTGCTTGGAAGAAGTCGTTGTCGGACCTC	0,5138
3362294AC33	A>C	33	TGCAGTTATGATGGGTTCTTCATCTTGGTCTACACCCCCACCAGCCTTTGGATCAGTCATGGGCTCTTC	0,4962
3364140TC30	T>C	30	TGCAGAGATCACACTCCTGAATGTGAAGTGTGTCGAAAAGAAGACCGAGATCGGAAGAGCGGTTCAGCA	0,4954
3587199TC18	T>C	18	TGCAGAGAAACGTACTCGTAGTCACGTGGACTCGGGTAGGAGACCAGCCGAGATCGGAAGAGCGGTTCA	0,4792
5036254TG16	T>G	16	TGCAGGAGATGCGGCTTCACGGGGTGGCGTGCCCCGAGGGAGCCCTCGTCGCCGCCATCTGCGCCTTCG	0,4785
3345994GT12	G>T	12	TGCAGCCAACGAGGGTCGATCTTCCGTACTATCCATGTCGTCGTCGGAAGCGTCAGACAAGCCGAGATC	0,4780

SUMMARY

1. DArTseq markers allowed construction of a saturated genetic map of rye based on RIL4 mapping population.
2. Mapping DArTseq and DArT markers with known localization allowed assigning the linkage groups to proper rye chromosomes.
3. Composite interval mapping enabled the identification of a QTL corresponding to pollen fertility restoration gene within RIL4.
4. Association mapping allowed the identification of markers associated with the trait of interest.

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The project is funded by MRiRW – POSTĘP BIOLOGICZNY W PRODUKCJI ROŚLINNEJ, nr 21