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 the 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 et al. 2000); however, the one mapped to 4R chromosome is considered as the most important as it explains most of the phenotypic variance of the trait. Thus, its introduction into parental forms that originated from European material and lacked the gene is required what could be achieved if linked markers are available. The aim of the study was the identification of molecular markers of Rf genes in rye with cms pampa using RIL4 biparental mapping population.

MATERIAL AND METHODS

The plant material consisted of 92 RIL4 individuals that originated from a biparental cross (maintainer (N) x restorer line with cms-P based on Iranian materials). RIL4 and parental lines were genotyped with DArTseq and silico DArT markers. Pollen fertility restoration of the RILs was evaluated indirectly via phenotyping BC1F4 materials derived via backcrossing of the cms-p maternal line and RIL4 lines using visual bonitation scale (Geiger, Morgenstern 1975). A genetic map was constructed under MultiPoint UltraDense software (http://www.multiqtl.com). The map was visualised in MapChart (Voorrips 2002). Association mapping was performed in TASSEL (Bradbury et al. 2007). Composite interval mapping was performed in Windows QTL Cartographer (Wang et al. 2012). Cluster analysis was performed in PAST (Hammer et al. 2001).

RESULTS

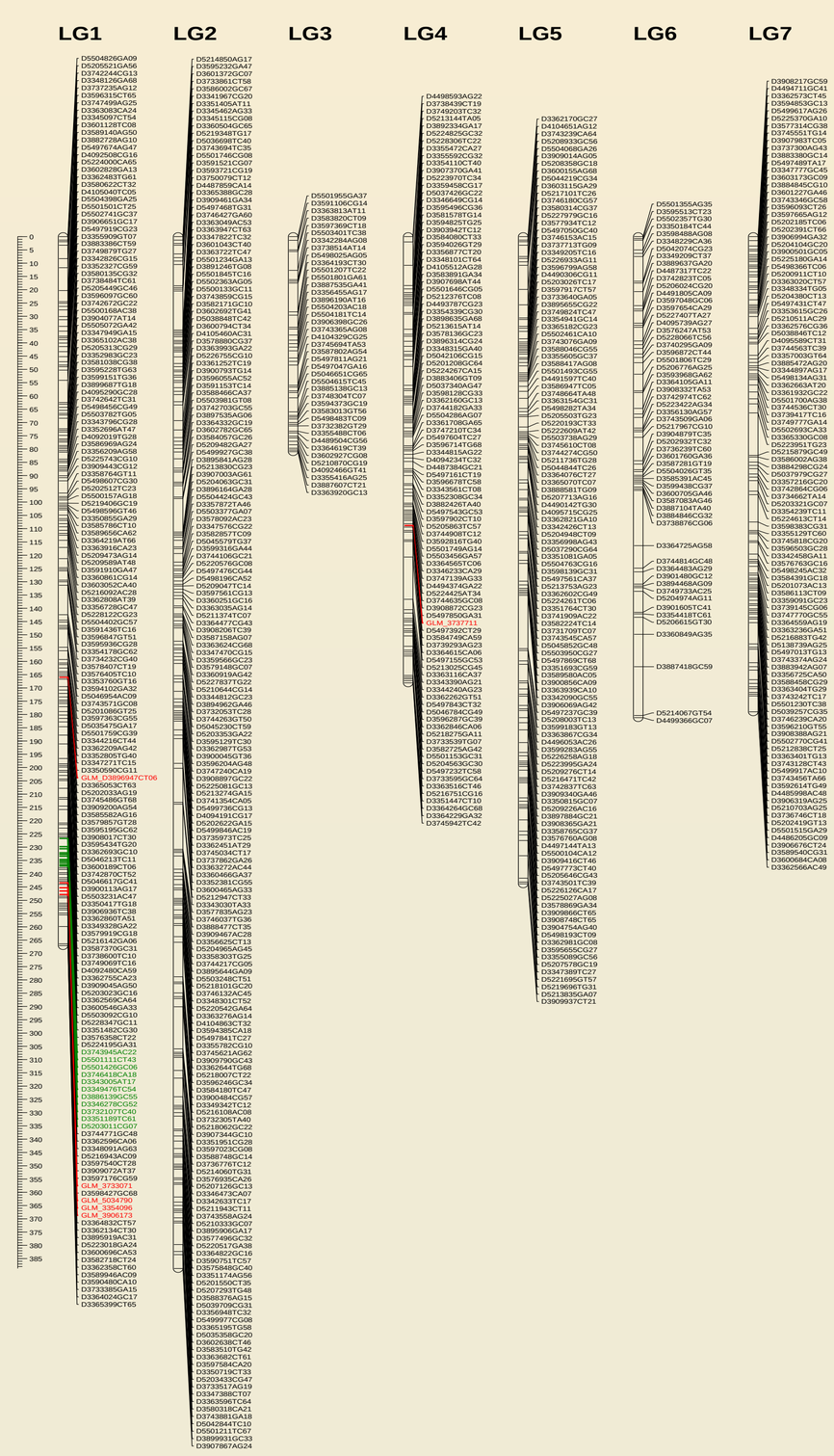
1.The BC1F4 population used for the evaluation of pollen fertility trait demonstrated that RIL4 materials exhibit a wide range of the trait variation. Both sterile, partly-fertile and fertile RIL4 lines (data not shown) encompass the mapping population. The genetic map consisted of 7 linkage groups covering 1516.43 cM. Out of 14 222 markers segregating in the population, only 790 were mapped with a few gaps spanning over 21 cM (Picture 1). DArTseq markers formed numerous groups of redundancy.

2.Composite interval mapping allowed the identification of a QTL (spanning over 3cM) with LOD function maximum equal to 5.9 within LG1 (covered 267.06 cM) explaining over 25% of the trait variance (Picture 2). The marker closest to the QTL was 0.6 cM from its maximum.

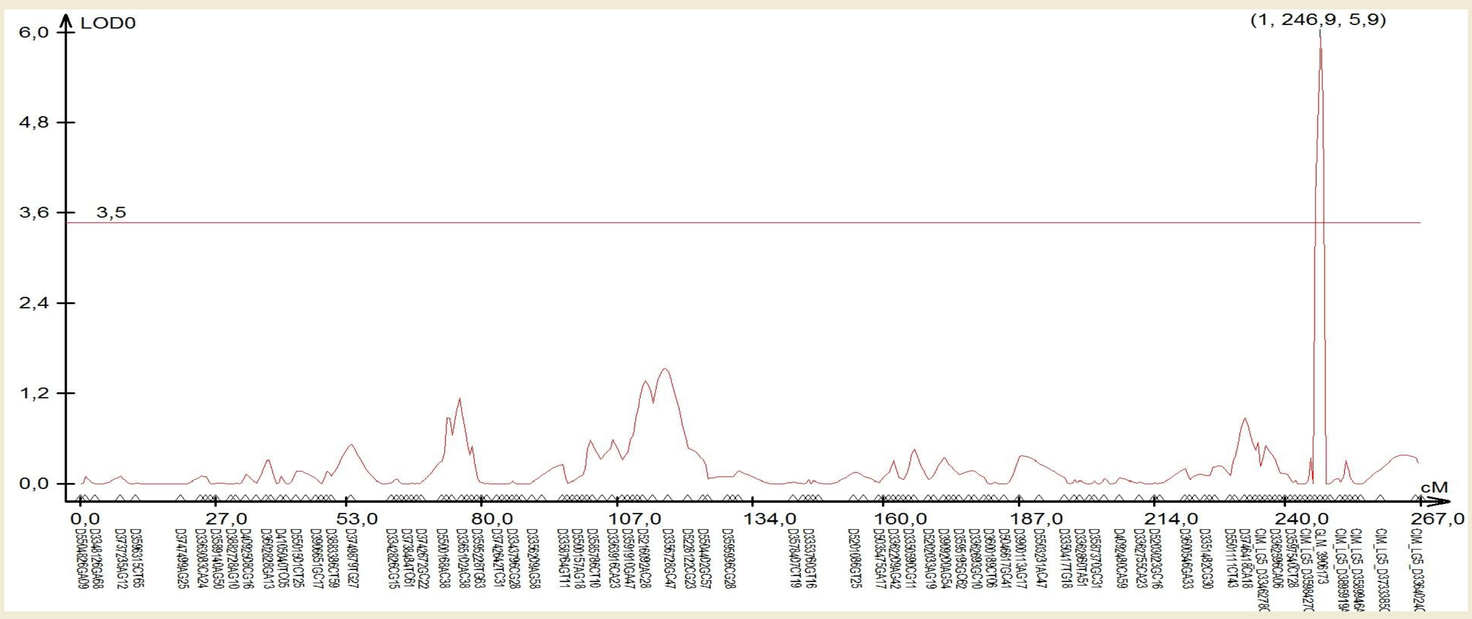
3.Additional markers were evaluated via association mapping (based on all available markers for the mapping population) and cluster analysis. There were 21 markers that passed Bonferroni test with association higher than 0.24. Based on cluster analysis, markers within the same group where the markers linked to the QTL found.

4.The markers forming LG1, associated markers and those identified based on cluster analysis (not shown) were used to remap LG1. The refined group contains 147 skeletons (plus 284 redundant ones) and covers nearly 250 cM. Composite interval mapping mapped the pollen fertility QTL between 221 and 240 cM (Picture 3). Its maximum was at 235 cM (LOD=4.3) and explained nearly 20% of phenotypic variance. The closest marker was 0.15 cM from its maximum. There were 19 skeletons with 87 redundant markers within QTL.

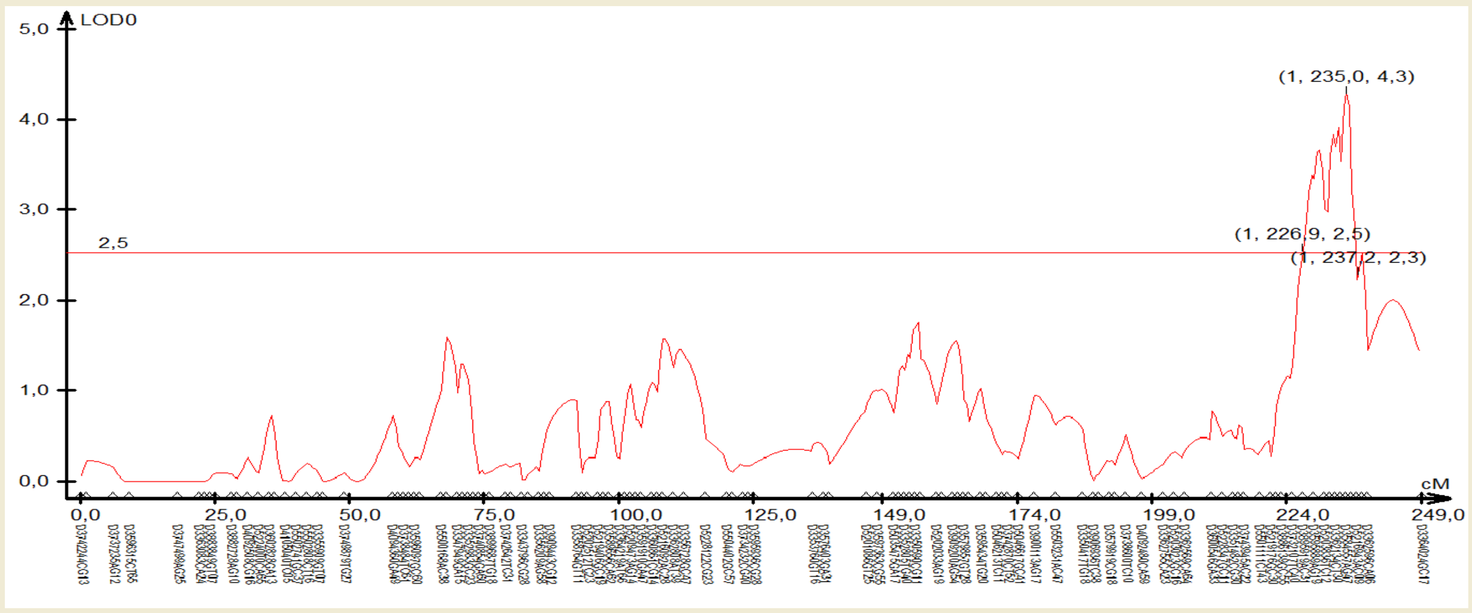
Picture 1. Illustration of the genetic map constructed based on RIL4 and DArTseq/silicoDArT markers. Markers in green were within the QTL region. Markers associated with the QTL are marked in red.



Picture 2. Composite interval mapping for LG1 (before remapping of group).



Picture 3. Composite interval mapping for LG1 (after remapping of group).



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SUMMARY

1. DArTseq markers allowed construction of saturated genetic map of rye based on RIL4 mapping population consisting of 92 lines.
2. Composite interval mapping enables the identification of a QTL corresponding to pollen fertility restoration gene within RIL population based on Iranian materials.
3. Association mapping allowed the identification of markers associated with the trait of interest.
4. Cluster analysis indicated that additional markers of the trait need to be taken into account.
5. When new map incorporating mapped markers and all additional once was constructed, the additional markers of the trait were within the very vicinity of the pollen fertility restoration QTL of the LG1.
6. Additional analysis are required to assign the LGs to rye chromosomes.

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