Genetic Analysis of Segregation Distortion of SSR Markers in F₂ Population of Barley

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Abstract

260 F₂ individuals derived from the cross between a photoperiod-thermo-sensitive genic male sterile (PTGMS) barley line C54S and a barley elite cultivar 98-26 were used to construct a genetic linkage map. Fifty-one out of total 191 SSR markers were mapped on the 7 chromosomes covering 772.4 cM of barley genome, with the average intervals of 15.1 cM. Among the 65 polymorphic SSR locus, 22 locus (33.8%) showed genetic distortion (P<0.05), and all of them deviated toward female parent C54S. The highest distortion was observed on chromosome 4H. The two putative segregation distortion regions (SDRs) were detected on chromosome 5H and 6H, respectively. Some potential factors involved in the segregation distortion were discussed in this study.

Keywords: Barley, F2 population, SSR markers, Segregation distortion

1. Introduction

Segregation distortion is a ubiquitous phenomenon in biosphere, which skews the frequency of alleles from their Mendelian expectations (Lu *et al.*, 2002). It was first reported in maize by Mangelsdorf and Jones (1926), and later many other crop species including rice (McCouch *et al.*, 1988; Zhang *et al.*, 2010), sorghum (Pereira *et al.*, 1994), tomato (Paterson *et al.*, 1988), coffee (Ky *et al.*, 2000), tobacco (Cameron and Moav, 1957), wheat (Loegering and Sears, 1963; Peng *et al.*, 2000; Kumar *et al.*, 2007). In barley, Segregation distortion was also reported many times (Heun *et al.*, 1991; Goloenko *et al.*, 2002; Li *et al.*, 2010). Segregation distortion is influenced by many factors, such as mapping population, gametophytic competition, abortion of the male or female gametes or zygotes, non-homologous recombination, transposable element and environmental agents et al (Kinoshita, 1993; Knox and Ellis, 2002; Yamagishi *et al.*, 2010). The development of high density molecular linkage maps provided a chance to survey the whole genome for loci showing distorted segregation (Harushima *et al.*, 1996; Causse *et al.*, 1994). In the present study, we constructed a barley genetic linkage map of F₂ population using SSR markers, assessed the frequency of segregation distortion occurrence, identified chromosomal regions consistently associated with segregation distortion in barley, and discussed the potential factors involved in the segregation distortion.

2. Materials and methods

2.1 Plant materials and DNA extraction

Two hundred and sixty individuals of F_2 generation derived from the cross between a photoperiod-thermo-sensitive genic male sterile (PTGMS) barley line C54S and a barley elite cultivar 98-26 were genotyped in this study.

Genomic DNA was extracted from freshly harvested leaves of the plants as described by Graner *et al.* (1990), with some modifications. The DNAs were detected by electrophoretic separation on 0.8% agarose gels. DNA concentration was adjusted to 50 ng/ μ l for PCR amplification.

2.2 SSR analysis

A total of 191 microsatellite (simple sequence repeats, SSR) primers covering the whole barley genome were screened for polymorphism between parents of the F_2 population, and 86 (45%) of them showed polymorphisms. Sixty well-separated polymorphic primers were selected for genotyping the F_2 population. SSR analysis was performed according to the methods of Chen *et al.* (1997) and Panaud *et al.* (1996). PCR was carried out in a PTC-225 thermocycler (MJ-Research, Watertown, MA, USA) with the following conditions: pre-denaturation at 94°C for 5 min; followed by 36 cycles of denaturation at 94°C for 45 s, annealing for 45 s (annealing temperature determined by primer pair sequence, usually 55°C), and extension at 72°C for 1 min; with a final extension at 72°C for 10 min. The amplified products were electrophoresed on 8% non-denaturing polyacrylamide gels or 6% denaturing polyacrylamide gels, and visualized by silver staining (Sanguinetti *et al.*, 1994; Bassam *et al.*, 1991).

2.3 Construction of genetic linkage map

Sixty SSR primers showing clear polymorphisms between the parents were selected for genotyping the F_2 population, and generated sixty-five polymorphic loci. Linkage analysis was performed with the program MAPMAKER/EXP 3.0 based on the segregation data (Lander *et al.*, 1987). The recombination frequency (%) was converted to genetic distance (centiMorgan, cM) by the function of Kosambi (1944).

2.4 Detection of segregation distortion

Segregation distortion was detected for genotype segregation deviating from the expected Mendelian ratio (1:2:1 for codominant markers and 3:1 for dominant markers in F_2 population). Chi-square test has been often used to reveal the deviation. In addition, the presence of a segregation distortion region (SDR) was identified when three or more closely linked markers exhibited significant segregation distortion in F_2 population. The most-skewed marker in a SDR was considered the most likely location of a distorting factor.

3. Results

3.1 Genetic linkage map of barley F_2 population

Among the 191 SSR markers, 86 markers (45%) showed polymorphisms between the parents (C54S/98-26). sixty well-separated polymorphic primers were selected for genotyping the F_2 population, and generated 65 polymorphic loci (including 44 codominant loci and 21 dominant loci). 51 out of totally 65 polymorphic SSR marker loci were located in the 9 linkage group, and mapped on the 7 chromosomes spanning a total length of 772.4 cM of barley genome, with the average intervals of 15.1 cM between adjacent markers. The marker distribution over the map was even except for several gaps on chromosomes 2H and 3H. Chromosomes 1H and 3H were each split into two linkage groups (Figure 1).

3.2 Segregation distortion and SDR

In the barley F_2 population, 22 (33.8%) out of the 65 polymorphic loci showed segregation distortion at the 0.05 level of significance, and 15 (23.1%) of them showed extremely significant deviation at the 0.01 level (Table 1). All the distorted loci were skewed towards the female parent C54S, while no markers deviated towards the male parent 98-26. Among 44 codominant loci, 14 (31.8%) significantly deviated from Mendelian segregation (P<0.05), and 9 (20.5%) had extremely significant deviation (P<0.01). However, among 21 dominant loci, more dominant loci (38.1%) showed segregation distortion at the P<0.05 level of significance, and 28.6% showed segregation distortion at the P<0.01 level of extreme significance. The highest distortion was observed on chromosome 4H (Table 1).

19 out of total 22 the distorted loci (at the P<0.05 level of significance) were mapped on these linkage groups. Markers with segregation distortion were not dispersed randomly among the 7 barley chromosomes. For example, A large proportion (47.4%) of mapped distorted markers were mapped to chromosome 5H and all showed extremely significant deviation (p<0.01), while no markers on chromosome 7H showed distorted

segregation (Table 1).

It can be observed from the map (Figure 1) that most distorted markers clustered in special regions on chromosomes. This is termed as Segregation Distortion Regions (SDR). Two SDRs were identified on chromosome 5H and 6H (SDR5 and SDR6, respectively). The size of SDR5 (97.1 cM) was much larger than that of SDR6 (45.4 cM). Both of two SDRs were found to be close to centromeric regions (Figure 1).

4. Discussion

In this study, distorted markers were clustered. A significant proportion (63.2%) of the mapped distorted markers was mapped to two SDRs. This result was similar to that reported by Li *et al.* (2010) who found that 72% of the distorted markers in their barley composite map were clustered. Other studies also showed distorted segregation were not evenly distributed over the barley genome but confined to a few distinct regions on chromosomes (Graner *et al.*, 1991; Thompson *et al.*, 1991; Devaux *et al.*, 1995; Steffenson *et al.*, 1995; Ramsay *et al.*, 2000; Marcel *et al.*, 2007). Some of the reported SDRs were located within the SDRs identified in the present study and were thus validated. For example, the SDR previously found by Steffenson *et al.* (1995) on chromosomes 5H was confirmed to be in the same region as that identified in this study. The fact that markers with segregation distortion are clustered in particular regions indicates that segregation distortion in the F_2 population is most likely caused by genetic factors and unlikely to be due to statistical bias, genotyping or scoring errors (Plomion *et al.*, 1995).

Furthermore, both two SDRs found in this study were in centromeric regions and may be due to genetic processes related to position near centromeres. The mechanism causing the centromeric effect on segregation distortion is unknown but understanding should now progress more rapidly with the development of high-throughput sequencing technology.

Segregation distortion varies significantly with population types (Song *et al.*, 2005). Xu *et al.* (1997) found that the rate of segregation distortion was lower in F_2 populations compared to other populations. However, a large proportion (33.8%) of polymorphic markers showed genetics distortion (P<0.05) in this study, and all distorted markers deviated toward the male sterile barley line C54S. The result is most likely related to the particular mapping parent. Zhang *et al.* (2006) reported that segregation distortion could be partially caused by gametophytic and sterile factors. Association between Regions of segregation distortion and QTLs or genes was also detected by Kintzios *et al.* (1994). In this study, Genetic linkage map of barley F_2 population was constructed, which could be used as a platform for map-based gene or QTL cloning. Further QTLs analysis of gametophyte gene and sterile gene will contribute to enhance our knowledge in the mechanism of segregation distortion, and allow breeders to predict the appropriate population size and have a good chance of getting the desired recombinants.

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Markers	Chromosome	Codominant	Genotype in the F ₂ population			2	Direction of all or of
		or dominant	A/A	Н	B/B	χ	Direction of skewed
Bmac0154	1H	Codominant	63	114	83	7.02*	C54S
GBM1278	1H	Dominant	177	0	82	5.76*	C54S
GBM1468	2Н	Codominant	53	125	82	6.85*	C54S
Bmag006a	3Н	Codominant	58	119	83	6.67*	C54S
Bmag006b	3Н	Dominant	174	0	83	6.87**	C54S
Bmag0353b	4H	Dominant	60	0	199	366.96**	C54S
Bmag0341	4H	Dominant	180	0	80	4.31*	C54S
scssr07106	5H	Codominant	40	130	90	19.23**	C54S
Bmag0323	5H	Codominant	19	121	120	79.72**	C54S
GBM1426	5H	Codominant	39	129	92	21.62**	C54S
GBM1506	5H	Codominant	40	127	93	21.75**	C54S
GBM1176	5H	Codominant	42	126	89	17.12**	C54S
GBM5028	5H	Codominant	35	127	98	30.67**	C54S
Bmac0163	5H	Codominant	17	131	112	69.44**	C54S
Bmag0751	5H	Codominant	16	124	115	75.68**	C54S
Bmag0113	5H	Dominant	44	0	216	8.62**	C54S
Bmag0496	6Н	Codominant	63	109	88	11.59**	C54S
HVM11a	6H	Dominant	130	0	130	85.34**	C54S
HVM11c	6H	Dominant	173	0	87	9.48**	C54S
Bmag0770	N	Codominant	48	130	82	8.89*	C54S
HVM54	N	Codominant	59	117	83	6.84*	C54S
Bmag0337a	Ν	Dominant	145	0	115	50.26**	C54S

Table 1. Chi-square test for segregation distortion of SSR markers in the F₂ population

Note: $\chi^2_{0.05,1}=3.84$; $\chi^2_{0.01,1}=6,63$; $\chi^2_{0.05,2}=5.99$; $\chi^2_{0.01,2}=9.21$. *and** indicate significant difference at 0.05 and 0.01 probability level, respectively. N indicates distorted markers which were not found on the linkage map.



Figure 1. Genetic linkage map of barley F₂ population and the distribution of markers with segregation distortion in the map. The numbers on the left are the genetic distances in centiMorgans (cM) between markers. Marker names are on the right. The *oval shapes* on the chromosome 5H and 6H indicate the position of centromeres. * and ** indicate significance levels of distorted segregation at 5% and 1%, respectively