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Construction of RFLP Linkage Map and Identification of QTLs Controlling Spikelet and Pollen Sterility in Rice*

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Abstract Quantitative trait locus (QTL) analysis has been carried out to identify genes conferring spikelet sterility and pollen sterility in rice. As a first step, an RFLP linkage map based on the recombinant inbred lines (RILs) was constructed. 125 RILs (F_{10}) derived from a cross between a *Japonica* cultivar Taichung 65 and an Indica cultivar ARC10313 were developed. The RFLP map contained 113 well-dispersed RFLP markers. Total map length was 1462.4 cM Linkage arrangement of the RFLP markers was in good agreement with that of the previously constructed maps. 63 backcross F_1 lines (BF_1) derived from RILs were used as a segregating population for QTL analysis. Three QTLs for F_1 spikelet sterility were detected on Chromosome 1, 7 and 11. Among them, a QTL near the RFLP marker R1789 (chromosome 7) was detected at significant level $P=0.0001$, and one QTL for F_1 pollen sterility was also detected near R1789 marker. Rf4 on chromosome 7 is known as fertility restorer gene. Above QTLs detected on chromosome 7 in this study appeared to correspond to Rf4 locus.

Key words RFLP; Spikelet sterility; Pollen sterility; QTL analysis; Rice

水稻 RFLP 连锁图谱的构建及控制小穗不育和花粉不育的 QTL 分析

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摘 要 用台中 65(粳稻)/ARC10313(籼稻)的重组近交系(F_{10})构建了 RFLP 连锁图谱，含 113 个分布均匀的标记。作成的图谱覆盖全基因组，全图总长 1462.4 cM，图中标记位置与所使用的参照图谱基本符合。利用该重组自交家系材料与亲本台中 65 回交得到 BF_1 家系，用于对小穗不育和花粉不育的 QTL 分析，检测出 3 个小穗不育和 1 个花粉不育 QTL，且有一个小穗不育位点和花粉不育位点重叠于第 7 条染色体的标记 R1789 处。已知的恢复基因 Rf4 与该座位对应。正在建立该座位的近等基因系以进行基因精确定位和克隆。

关键词 水稻；RFLP；小穗不育；花粉不育；QTL

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RFLP markers are used in distantly related crosses and quantitative trait loci (QTLs) can be normally detected in the mapping population. With RFLP markers, well-saturated linkage map based on recombinant inbreed lines (RILs) of rice was constructed in this paper. Sterility is a major barri-

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er for utilizing alien germplasm for rice breeding[2~4]. Varying degrees of hybrid sterility are commonly seen in crosses between *Indica* and *Japonica* varieties. Using the RILs and their backcross lines, the QTLs associated with spikelet sterility and pollen sterility in rice was sought.

1 Materials and methods

1.1 Construction of RFLP linkage map

125 F_{10} lines developed by SSD (single-seed-decent) were planted and DNA of these RILs was extracted for RFLP linkage map construction. RFLP probes (Harushima 1998, Tusnematus 1996[1, 6]) were supplied by Rice Genome Program, Japan. RFLP between the parents were surveyed using six enzymes (*Bam*HⅠ, *Bgl*Ⅱ, *Dra*Ⅰ, *Eco*RⅠ, *Eco*RⅤ and *Hind*Ⅲ). 113 markers were selected to construct the linkage map.

1.2 Spikelet sterility observation

Indica cultivar ARC10313 was crossed to a *Japonica* cultivar Taichung65 as the female parent. 63 RILs were backcrossed to Taichung65 in 1999 and the 63 backcross F_1(BF_1) lines were planted in 2000. Spikelet sterility was calculated as the percentage of empty spikelets over total spikelets per panicle for each of the RILs and BF_1.

1.3 Pollen sterility observation

28 RILs were crossed to Taichung65 in 2000 and these backcross F_1(BF_1) lines were planted in 2001. With BF_1 in 2001, pollen sterilty was observed from anthers collected from spikelets at 1 to 2 days before anthesis and stored in 70% ethanol and estimated as the percentage of pollen grains that could be stained with acetic carmine. QTL analysis was conducted using QGENE (Nelson 1997), a software for DNA-marker-based genetic analysis on Macintosh[5].

2 Results

2.1 Construction of RFLP linkage map

The RFLP map based on the RILs was shown in Fig. 4. It included 113 well-dispersed RFLP markers. Since markers closely linked to the terminal ends in the previous maps (Harushima 1998, Tsnematsu 1996) were used in this study, the present map covered whole genome. Total map length was 1462.4 cM. Linkage of the RFLP markers was in good agreement with that of the previously constructed maps[1, 6].

2.2 Spikelet sterility

Among the RILs, spikelet sterility was found and the frequency distribution was shown in Fig. 1. Though 4 QTLs for RIL sterility were detected, the effects of the QTLs were little (Table 2), therefore the spikelet sterility maybe resulted from F_1 steriltiy.

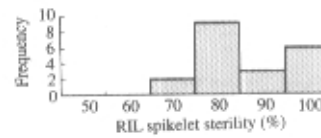

Fig. 1 Frequency distribution for RIL spikelet sterility

Frequency distribution for BF_1 spikelet sterility in 2000 was shown in Fig. 2. Three QTLs for BF_1 spikelet sterility were detected on Chromosome 1, 7 and 11 using BF_1 population (Fig. 4, Table 3). ARC10313 alleles in these regions reduced the spikelet fertility on the heterozygous conditions. Among them, the QTL near the RFLP marker R1789 (chromosome 7) was detected at significant level $P = 0.0001$. In the heterozygous condition, ARC10313 allele around the RFLP marker R1789 caused low spikelet sterility by 9.4%.

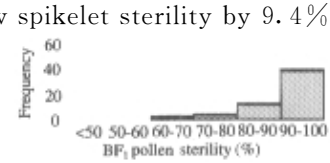

Fig. 2 Frequency distribution for BF_1 spikelet sterility

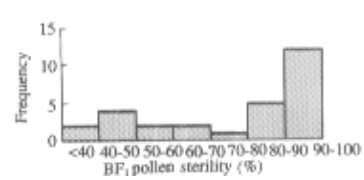

Fig. 3 Frequency distribution for BF_1 pollen sterility

2.3 BF_1 pollen sterility

Frequency distribution for BF_1 pollen sterility in 2001 was shown in Fig. 3. One QTL for F_1 pollen sterility were also detected near R1789 marker on Chromosome 7 (Fig. 4, Table 4) using BF_1 population in 2001. ARC10313 allele in this region reduced the pollen fertility on the heterozygous condition by 10.7%.

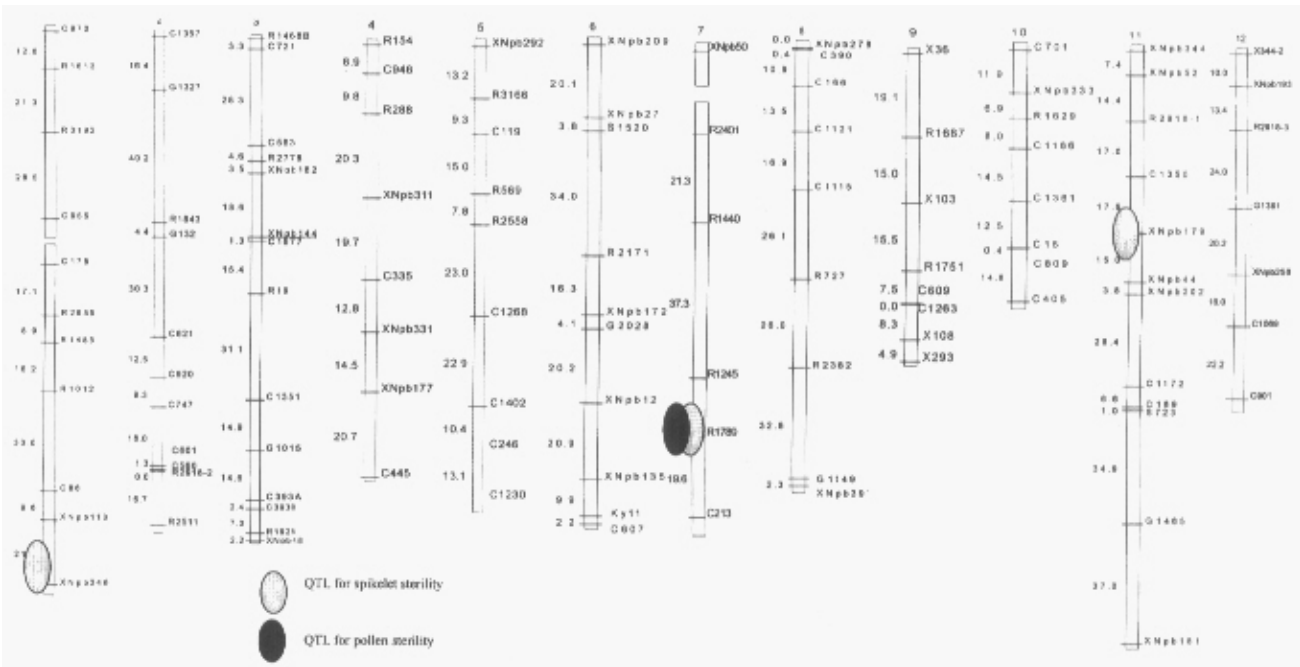

Fig. 4 An RFLP map based on the recombinant population (Taichung65/ARC10313)

3 Discussions

The linkage map presented here can be applied to analysis other agronomic traits as it covers adequately the rice genome. Strong segregation distortion to ARC10313 was observed in chromosome 3, 6, 9, 10 and 11. At the Ky11 and C607 loci, less than 10% of RIL plants were homozygous for Taichung65 alleles. The segregation distortion may cause a problem as it will be difficult to eliminate ARC10313 alleles from these regions.

The QTL near R1789 on Chromosome 7 was detected not only conferring spikelet sterility but also pollen sterility in this study. Rf4 (Zhang *et al.* 1997) on chromosome 7 is known as fertility restorer gene. QTL detected in this study appeared to correspond to this fertility restoration locus. This sterility QTL was the first one detected using the RILs derived from *Indica*/*Japonina* variety cross. More BF_1 lines and near-isogenic lines are now under construction for further QTL analysis and gene mapping.

Table 1 RFLP loci showing segregation distortion

RFLP loci(Chr.)	AA[1)]	aa[2)]	Total	χ^2	RFLP loci(Chr.)	AA[1)]	aa[2)]	Total	χ^2
C563(3)	23	97	120	45.63	C607(6)	8	112	120	90.13
R2778(3)	14	109	123	73.37	R2638(9)	49	74	123	5.08
XNpb182(3)	14	110	124	74.32	C609(9)	49	75	124	5.45
XNpb144(3)	29	94	123	34.35	C1263(9)	49	75	124	5.45
C1677(3)	27	91	118	34.71	XNpb108(9)	46	74	120	6.53
R19(3)	46	74	120	6.53	XNpb293(9)	44	74	118	7.62
XNpb48(3)	49	75	124	5.45	XNpb333(10)	42	79	121	11.31
XNpb27(6)	39	79	118	13.55	R1629(10)	38	73	111	11.03
S1520(6)	46	72	118	5.72	C1166(10)	47	74	121	6.02
XNpb12(6)	48	74	122	5.54	XNpb52(11)	36	70	106	10.90
XNpb135(6)	25	97	122	42.49	S723(11)	41	62	103	4.28
Ky11(6)	5	120	125	105.80	G1465(11)	94	21	115	46.33

1) AA: Number of plants with TC65/TC65 genotype; 2) aa: Number of plants with ARC10313/ARC10313 genotype; ($\chi^2_{(1,0.05)}=3.84$)

Table 2 QTLs detected for RIL spikelet sterility

RFLP loci Near QTL	Chromosome	AA[1)]	(N)	aa[2)]	(N)	F	P	Additive[3)]
S1520	chr6	95.93	45	92.79	72	7.38	0.0076	1.8
XNpb27	chr6	95.9	39	93.04	78	5.61	0.0195	1.4
C955	chr1	95.07	74	92.48	47	5.23	0.024	1.3
R727	chr8	95.4	55	93.08	62	4.43	0.0375	1.2

1) AA: homozygous for Taichung65 allele. 2) aa: homozygous for ARC10313 allele. 3) Additive: Additive effect of Taichung65 allele.

Table 3 QTLs detected for BF_1 spikelet sterility

RFLP loci Near QTL	Chromosome	AA[1)]	(N)	Aa[2)]	(N)	F	P	Additive[3)]
R1789	chr7	93.36	34	83.91	18	17.93	0.0001	9.4
XNpb179	chr11	92.06	26	87.2	28	4.2	0.0456	4.8
XNpb346	chr1	91.64	38	87.05	18	4.15	0.0466	4.6

1) AA: homozygous for Taichung65 allele. 2) Aa: heterozygote. 3) Additive: Additive effect of homozygous allele.

Table 4 QTLs detected for BF_1 pollen sterility

RFLP loci Near QTL	Chromosome	AA[1)]	(N)	Aa[2)]	(N)	F	P	Additive[3)]
R1789	chr7	88.71	14	67.25	14	8.96	0.006	10.7

1) AA: homozygous for Taichung65 allele. 2) Aa: heterozygote. 3) Additive: Additive effect of homozygous allele.

References

[1] Harushima H, M. yano, A. shomura *et al*. A high-density rice genetic linkage map with 2275 markers using a single F_2 population. *Genetics*, 1998, 148: 479～494

[2] Ikehashi, H, H. Araki *et al*. Genetics of F_1 sterility in the remote crosses of rice. *Proceeding of the international Rice Genetics Symposium*. IRRI, Manila Philipines, 1986, 119～333

[3] J. Wan, Y. yamaguch, H. kato, *et al*. Two new loci for hybrid sterility in cultivated rice (*Oryza sativa* L.) *Theor. Appl. Genet*, 1996, 92: 183～190

[4] K. Doi, K. Taguchi, A. yoshimura *et al*. A new locus affecting high F_1 pollen sterility found in backcross progenies of *Japonica* rice and Africa rice. *Rice Genetics Newsletter*, 1998, 15: 149～151

[5] Nelson, J C *et al*. QGENE: software for marker-based genomic analysis and breeding. *Molecular Breeding*, 1997, 3: 239～245

[6] Tsunematsu H, Yoshimura A., Harushima Y., *et al*. RFLP framework map using recombinant inbred lines in rice. *Breeding Science*, 1996, 46: 279～284

[7] Zhang T S, Bharaj Y, Lu S S *et al*. Mapping of the Rf-3 nuclear fertility-restoring gene and RFLP markers. *Theor Appl Genet*, 1997, 94: 27～33