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AFLP and RFLP linkage map in *Coix*

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Abstract

Coix is a genus in the grass family placed in the tribe *Maydeae*. It is closely related to maize and is also used as a crop plant. Since many valuable traits have been identified recently in *Coix*, it is considered to be a valuable genetic resource, particularly for maize improvement. In this study, a *Coix* genetic linkage map was constructed using an F_2 population of 131 individuals. Eighty AFLP and 10 RFLP markers were mapped, covering a total length of 1339.5 cM with an average interval of 14.88 cM. The map consisted of 10 linkage groups, were consistent with the chromosome numbers observed cytogenetically. Both AFLP and RFLP markers were used first for genetic analysis in *Coix*. AFLP markers were generated by two restriction enzyme combinations, EcoRI/MseI and PstI/MseI. A total of 1349 bands were amplified, of which 140 were polymorphic. The polymorphism detection efficiency of the two enzyme combinations was compared, and utility of AFLP markers to construct the linkage map was discussed. Ten RFLP markers detected by three different probes were distributed on eight different linkage groups. The results provide a foundation to map and isolate important genes in *Coix*, and to investigate its genomic architecture, possible origins, and relationship with maize at the DNA level.

Introduction

Coix lachryma-jobi L. is a member of the grass family in the tribe *Maydeae*. It has been cultivated as a crop in China for thousands of years, but is little characterized genetically. Recently, some valuable traits were identified in *Coix* germplasm resources, such as high protein and oil in grain, long duration of stay-green, resistance to maize diseases, etc. (Huang 1995). The construction of a *Coix* genetic linkage map is essential for the location of such trait loci with molecular markers in order to facilitate their manipulation in its improvement. In addition, *Coix* is the closest relative to the Old World genus *Zea*. It is the only Asiatic genus ever seriously considered as a possible ancestor of maize (Hallauer and Miranda 1981). Even now, the origin of maize is not fully understood. Most of the study on this question focused on the *Teosinte* and *Tripsacum* genera, which both occur in the New World. It remains unclear if *Coix* made any contribution to the origin and evolution of maize. Studies on *Coix* molecular genetics may shed some light on this issue.

Karyotype formulae of three species and four varieties of *Coix* were investigated (Zhuang et al. 1994), and are consistently $2n = 20$. So far only RAPD markers have been used to investigate genetic diversity and relationships in *Coix* accessions (Li et al. 2001). AFLP technology provides a robust method for detecting a large number of polymorphisms from a single PCR reaction within

a very short period (Vos et al. 1995). Thus, the AFLP approach was widely used to rapidly create linkage maps in a variety of plant species (Castinglioni et al. 1999; Maheswatan et al. 1997; Alonoso-Blanco et al. 1998; Lu et al. 1998). On the other hand, RFLP technology offers an alternative approach to construct genetic linkage map in plants (Helentjaris et al. 1986). This molecular marker technique is more reliable and has better repeatability even though it is more laborious and costly. One important advantage of RFLP is that complementary DNA probes can be cross-mapped to provide anchors that allow genomes to be compared among different plant species (Gale and Devos 1998). Over the past 15 years the relationships were investigated using molecular marker methods among the genomes of almost all economic grass crops, but *Coix* was not included among them. Thus, the objective of this investigation was to construct a genetic linkage map with AFLP and RFLP markers using an F_2 population in *Coix*.

Materials and methods

Plant materials

An F_2 population was generated through a cross between BJ and WH *Coix* accessions. There was a relatively large genetic diversity between the two parents observed by RAPD assay (Li et al. 2001). F_1 plants were self-pollinated to develop an F_2 population. Ultimately, a mapping population with 131 individuals was obtained.

Molecular marker assay

Genomic DNA was extracted according to Saghai-Maroof et al. (1984). The AFLP protocol was carried out as described by Vos et al. (1995). Genomic DNA was digested with a combination of EcoRI/MseI or PstI/MseI restriction enzymes. The adapter sequences for all the enzymes were synthesized according to Vos et al. (1995). They were coded as Ead-1, Ead-2, Mad-1, Mad-2, Pad-1, and Pad-2, respectively. Selective amplification was conducted using primers with two or three selective nucleotides. Eight primers for each enzyme-sequence were synthesized. PCR products were electrophoresed onto 6 % denaturing polyacrylamide gel and were visualized using the silver-nitrate staining method as described by Tixier et al. (1997). Only clear and unambiguous AFLP fragments ranging from 100 to 800 bp were scored. AFLP markers were named according to the two primers and the fragment molecular weight. RFLP analysis was conducted as described by Gardiner et al. (1993) with a few modifications, i.e., the amount of the probe (200 ng instead of 100 ng) and Klenow enzyme (4 unit instead of 2 unit) were doubled in order to increase the signal strength. Total DNA was digested by four restriction enzymes, BamHI, EcoRI, EcoRV, and HindIII respectively. A total of 79 genomic or cDNA probes from maize, rice and barley respectively were used for RFLP.

Genetic map construction

RFLP markers were codominantly scored while AFLP markers were dominantly scored. Linkage analysis was carried out using Mapmaker 3.0. Markers were grouped with the two-point group command at LOD = 3.0 and maximum recombination threshold of 0.25. The Kosambi mapping function was used to convert recombination frequencies into map distances (Kosambi 1994).

Results and analysis

AFLP analysis

In this study, from 64 primer combinations (PC) with EcoRI/MseI and PstI/MseI, the most informative nine PC for EcoRI/MseI and eight PC for PstI/MseI were selected for the segregation analysis in a mapping population. The visible band numbers and polymorphisms generated by each of these 17 PC were scored. For the EcoRI/MseI enzyme combination, polymorphism ranged from 3.8 % to 17.6 % for individual PC with an average of 7.03 %. The polymorphism for PstI/MseI varied between 10.1 % and 22.9 %, with an average of 14.62 % per PC. Totally, 1349 bands were amplified, including 754 bands by EcoRI/MseI and 595 by PstI/MseI. Among all amplified bands, 140 bands were polymorphic, including 53 bands from EcoRI/MseI and 87 bands from PstI/MseI, respectively. The percentage of informative loci

Table 1. Polymorphisms between parents screened by RFLP markers from different origins in *Coix*.

Probe sources	Polymorphic probes	Non-polymorphic probes	No signal probes	Total probes	Polymorphism (%)
Maize genomic	12	17	5	34	37.5
Maize cDNA	6	16	2	24	25.0
Barley cDNA	2	6	3	11	18.2
Rice cDNA	4	4	2	10	40.0
Total	24	43	12	79	30.4

Table 2. χ^2 test for genotypic segregation based on RFLP markers in F_2 population.

Probes	Name	Gene Bank No.	A	H	B	D	χ^2	$\chi^2_{0.05}$	$\chi^2_{0.01}$
A2	UMC157	–	20	39	16	–	0.209	5.99	9.30
G6	UMC168	–	19	72	32	–	6.31	5.99	9.30
J6	UMC44a	–	28	74	20	–	6.60	5.99	9.30
2A4	csu29	T12 666	33	60	30	–	5.65	5.99	9.30
2D11	csu109	T12721	24	59	34	–	1.71	5.99	9.30
2D12	csu110	T12722	–	–	35	85	0.90	3.84	6.63
2E2*	csu116	M95072	76	45	7	–	81.56	5.99	9.30
2G9a	UMC8	–	15	58	44	–	7.45	5.99	9.30
2G9b	UMC8	–	–	–	32	86	0.237	3.84	6.63
R5*	BCD207	–	58	52	11	–	50.0	5.99	9.30
R62*	RZ244	AA231738	13	36	75	–	90.133	5.99	9.30
R64	RZ261	AA23165	24	52	40	–	6.31	5.99	9.30
R65	RZ296	AA23174	25	41	15	–	2.52	5.99	9.30

*Denotes the observed data deviate from theoretical expectation at $P \leq 0.01$ level.

was significantly different between the EcoRI/MseI and PstI/MseI combinations. The average polymorphism of each PstI/MseI PC was twice that of each EcoRI/MseI PC, indicating that in *Coix* the former are more efficient in detecting polymorphism. This result is similar to those reported by other researchers for other plants (Eujaye et al. 1998; Maheswaran et al. 1997). In addition, the profiles generated by PstI/MseI PC were clearer and easier to score because there were fewer bands per gel and reduced background. Among 140 AFLP markers, 64 were WH-specific and 76 were BJ-specific. According to a χ^2 test, only 15 of 140 AFLP loci deviated from the expected Mendelian segregation ratio of 3 : 1 at $P < 0.01$. The distortion segregation percentage for AFLP markers was 10.7 %.

RFLP analysis

Thirty-four maize genomic probes, and 24 maize, 11 barley and 10 rice cDNA probes were screened for polymorphism between the two parents. Only 12, 6, 2, and 4 probes, respectively were polymorphic (Table 1). The polymorphism for each type of probe was 37.5 %, 25.0 %, 18.2 %, and 40 %, respectively with an average of 30.38 %. Rice cDNA probes had the highest polymorphism, followed by maize genomic probes which had much higher polymorphism than maize cDNA probes. Consider-ing the resultant quality of hybridization, cDNA probes would be better than genomic DNA probes for linkage map construction in *Coix* using RFLP probes from other crops. Thirteen polymorphic probes were used for segregation analysis. Except one maize probe (UMC8) that detected two loci, the other probes only detected one locus, thus a total of 14 loci were detected, of which five deviated from the expected segregation (1:2:1) at $P < 0.01$ (Table 2).

Linkage analysis

For construction of a genetic linkage map 140 AFLP and 14 RFLP polymorphism markers were used. With LOD = 3.0, two-point linkage analysis revealed that 95 markers were assigned

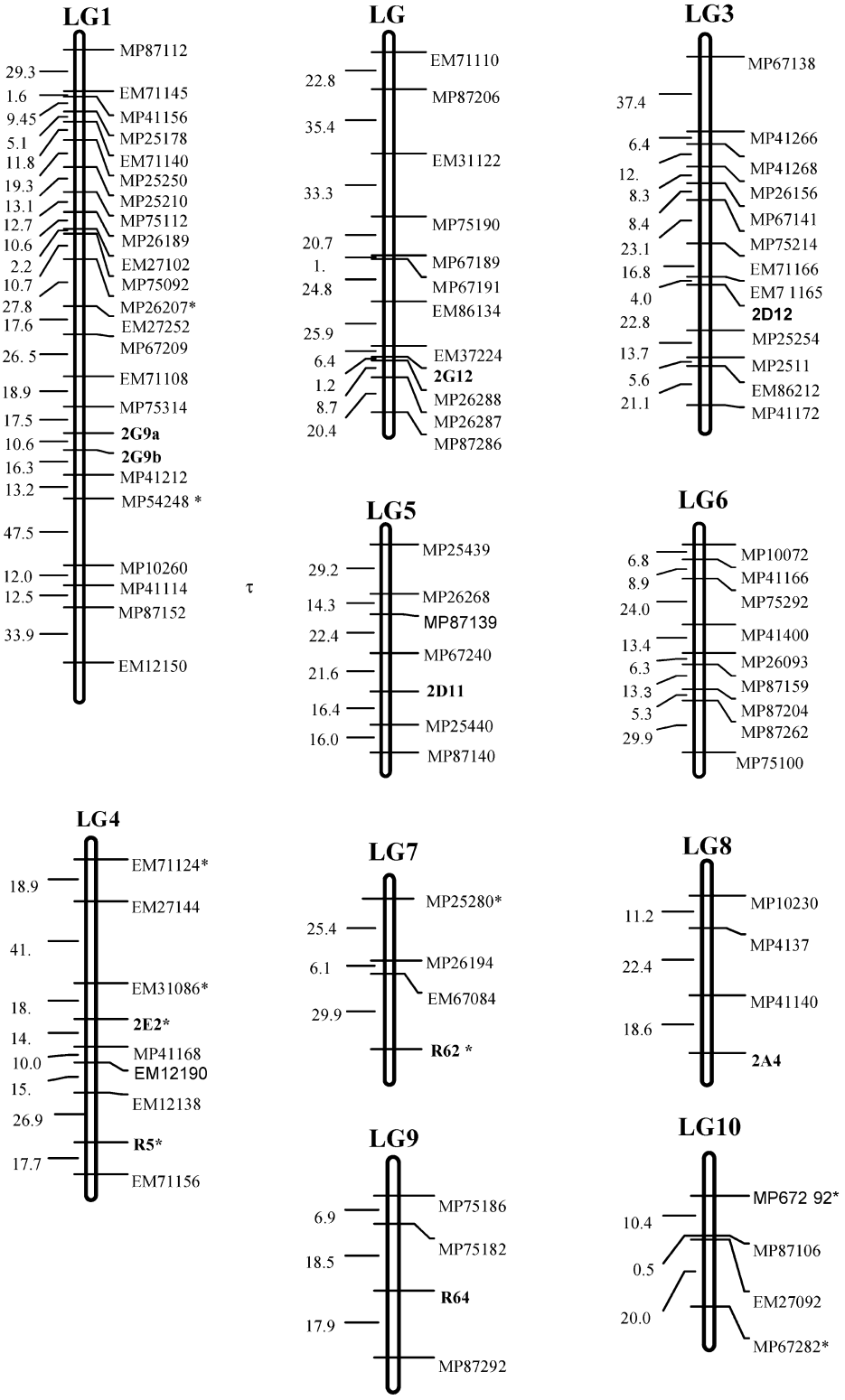

Figure 1. Genetic linkage map of *Coix*.

to 12 groups, two of which only contained two or three markers. With the LOD value elevated to 3.5, these two groups disappeared. Thus the genetic linkage map included ten linkage groups comprising 80 AFLP and 10 RFLP markers. It covered 1339.5 cM with average interval of 14.88 cM (Figure 1). Ten linkage groups were named by their linkage length. Linkage group1 (LG1) had the largest number of markers (24 markers) with the longest genetic distance (379.9 cM), while LG7, LG8, LG9, and LG10 each had only four markers, with 61.5, 52.1, 43.3, 30.9 cM respectively. LG2 and LG3 containing 12 and 13 markers covered distances of 201.0 and 179.5 cM, respectively.

All markers, either AFLP or RFLP, were randomly distributed on the map. EcoRI/MseI and PstI/MseI markers were mingled well together. Ten RFLP markers were located on eight different linkage groups, indicating RFLP markers could be well distributed among AFLP markers. It should be noted that four distorted segregation markers were located in LG4, which accounted for 40.0 % of total distorted loci on the linkage map (Figure 1).

Discussion

So far, this is the first reported genetic linkage map of *Coix*. It provides a foundation to map and isolate important genes in *Coix*, exploit the genomic architecture of *Coix*, investigate its possible origins, and compare the relationship between *Coix* and maize at the DNA level. The 10 RFLP markers distributed on eight different linkage groups offered anchors to construct higher density genetic maps in *Coix* for further comparative genetic studies.

Xiong et al. (1998) found that when LOD $\geq$ 3.0, it was difficult for many AFLP markers to be established in linkage groups. If the LOD value was lower, these unlinked markers were forced into groups. Thus the total genetic distance would be greatly extended. In this study, this phenomenon was also observed. With LOD = 3.0, 60 AFLP markers were not assigned to any linkage groups. On the other hand, the high number of unlinked markers in this investigation also reflects the need for many more markers to evenly cover the *Coix* genome, and for strong anchor marker information to improve mapping efficiency. Nevertheless, the segregation information for unlinked markers is still valuable for further mapping because *Coix* has strong tillering ability and can be kept in a greenhouse during winter, so this F_2 population could be permanently used.

In this investigation, a total of 79 RFLP probes from maize, rice and barley respectively were screened for polymorphism between parents (Table 1). Among them, 12 probes (15.2 %) gave no hybridization signal under high stringency washing conditions (65 °C, 0.2× SSC, 0.1 % SDS), while in other comparative mapping research (Ahn and Tanksley 1993; Kevin et al. 1999), this ratio is lower under moderate stringency washing conditions (65 °C, 1× SSC or 0.5× SSC, 0.5 % SDS). If the ratios of genomic and cDNA probes were compared, it was found that cDNA probes were more homologous among these plants, which is consistent with the microsynteny results that gene sequences are most conserved after evolution. Furthermore, most probes screened for polymorphisms can only detect one segregating locus in *Coix*, while exhibiting 2–4 loci in maize, indicating that the genome complexity of *Coix* is simpler than maize. That may be a useful message for gene cloning and manipulation. Considering polymorphism of different resource probes, it was also found that cDNA probes from rice gave highest polymorphisms between the parents. Since more than 200 rice and barley cDNA probes were used for maize comparative studies, and their location on the maize genome are known (Ahn and Tanksley 1993), it is necessary to use these probes for further comparative mapping.

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