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Genetic analysis of RAPDs in *Chamaecyparis obtusa* using partial diallel test

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Abstract: To develop molecular markers for *Chamaecyparis obtusa*, the inheritance of RAPD fragments was studied in diploid tissues of 5 parents and 30 diploid controlled F_1 progenies in each partial diallel crosses of these parents. Of 46 primers tested, 18 primers yielded 135 reproducible fragments, of which 42 fragments (31%) were polymorphic among the parents. Fourteen fragments amplified from 7 primers were selected to test the segregation among controlled F_1 progenies. All fragments observed in the parents were found in the progeny. Segregation of all variable fragments observed in diploid materials fitted the proportions expected for a dominant Mendelian trait. The segregating fragments were either present or absent confirming the dominant character of RAPD variation. The usefulness of RAPD fragments as genetic markers for estimating genetic diversity was also discussed. [En, 1 fig. 2 tab. 18 ref.]

Key words: *Chamaecyparis obtusa*; diallel crosses; RAPD; segregation analysis
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Random amplified polymorphic DNA (RAPD) is based on the polymerase chain reaction (PCR), using short arbitrary primers to amplify at random genomic fragments paired with the primers[1]. PCR can amplify a very small amount of DNA with no requirement for laborious cloning, nucleotide sequencing or Southern blotting. The amplified fragments can be detected by agarose gel electrophoresis within several hours. Despite obvious advantages and the continuously increasing number of studies employing RAPDs, some problems have been encountered with the use of RAPD markers. First, there are sometimes large numbers of segregation distortions with RAPD markers. Genetic studies with RAPDs have implied that only 33% of polymorphic DNA fragments segregated in a Mendelian fashion in *Picea abies*[2], approximately 69% in *Picea glauca*[3], and 86% in *Populus*[4]. In the diallel analysis of F_1 progenies of conifers, Carlson *et al.*[5] found that most but not all RAPDs were inherited in the expected dominant

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way. Halward *et al*.[6] found that normally dominant F_2 segregation occurred and the band patterns in peanut were often too complex to be used in genetic mapping. These studies indicate that some amplified DNA fragments do not comply with the simple dominant inheritance patterns. Hence, the employment of RAPD as a genetic marker may lead to draw a wrong conclusion when all polymorphic DNA fragments are scored as present or absent without regard to mode of inheritance. Secondly, the banding patterns of RAPD vary extensively under different amplification conditions, such as the concentration of primer relative to the template and the magnesium concentration (so-called artifactual variation)[7]. Therefore, the standardization of an optimal approach and internal control is necessary if the amplification products generated by RAPD are expected to be reproducible, scored confidently and resolved consistently between separate amplification reactions or between studies. The appearance of artifactual variation in RAPD banding patterns, as well as the segregation distortion, exemplifies the importance of genetic analysis of RAPD fragments prior to being used as genetic markers.

In *Chamaecyparis obtusa*, one of the most commonly planted forest tree species in Japan, allozyme has been used successfully as genetic markers. However, the number of allozyme loci currently studied in *C. obtusa* are limited to eleven to fourteen[8]. In contrast, RAPD markers allow direct access to the coding and non-coding regions of the genome, making their number potentially unlimited. Here, we report results from a study on the inheritance of RAPD fragments in unrelated individuals of *C. obtusa* and their F_1 progeny from a partial diallel cross. It was our intention to use this partial diallel design to determine (1) whether the polymorphic RAPD fragments follow the expected mode of dominant Mendelian traits and (2) whether the polymorphic fragments were actually observed in every expected F_1 progeny. We also discuss the possibility of applying these fragments as markers to the estimation of genetic diversity in managed *C. obtusa* forests.

1 Materials and methods

1.1 Plant materials

Partial diallel crosses between five plus-trees of *C. obtusa* originating from different parts of central Japan were established in the Forest Technology Center of Shizuoka Prefecture in 1987. Materials for this study came from these plus-trees and their partially diallel crosses F_1 progenies. The controlled F_1 progeny materials included six different crosses and their reciprocal crosses shown as '○' in Table 1. Dormant needles were collected from 15 individuals in each cross. All materials were stored at −20 ℃ until DNA extraction.

1.2 DNA extraction

Total cellular DNA was extracted from each individual by modified CTAB method[9,10]. Approximately 50 mg of frozen tissue was ground to fine powder in liquid nitrogen. The frozen powder was homogenized at 4 ℃ in 10 volumes of pre-cooled isolation buffer [100 g·L^{-1} polyethylene glycol (Mr 6 000), 0.35 mol sorbitol, 0.1 mol Tris (pH 8.0), 5 g·L^{-1} spermidine, 5 g·L^{-1} spermine. 5 g·L^{-1} 2-mercaptoehanol]. After centrifuged at 15 000 r·min^{-1} for 10 minutes at 4 ℃, the supernatant was removed. The pellet was homogenized with an additional 5 volumes of lysis buffer [0.35 mol sorbitol, 0.1 mol Tris (pH 8.0), 5 g·L^{-1} spermidine, 5 g·L^{-1} spermine, 50 g·L^{-1} 2-mercaptoethanol] and 1/10 volume of 100 g·L^{-1} sarcosine, mixed well and then incubated for 10 minutes at room temperature. An equal volume of 2× CTAB [20 g·L^{-1} cetyltrimethylammonium bromide, 0.1 mol Tris (pH 9.5), 20 mmol EDTA, 1.4 mol NaCl and 5 g·L^{-1} 2-mercaptoethanol] was added and the mixture was incubated at 65 ℃ for 10 minutes. The mixture was emulsified in

Table 1 List of controlled crosses used for analysis of RAPD variation

♀/♂	Izu 3	Izu 5	Fuji 1	Fuji 5	Fuji 6
Izu 3	-	○†	○	○	○
Izu 5	○	-			○
Fuji 1	○		-		○
Fuji 5	○			-	
Fuji 6	○	○	○		-

Notes: ♀ is female parent; ♂ is male parent; † ○ is F_1 materials selected for segregation analysis

an equal volume of chloroform-isoamyl alcohol (24∶1) and centrifuged at 15 000 r·min^{-1} for 10 minutes at room temperature. The supernatant was transferred and DNA was precipitated with an equal volume of ice-cold isopropanol. After centrifuged at 6 000 r·min^{-1} for 5 minutes at 4 ℃, the DNA pellet was air-dried and re-dissolved in a small quantity of TE [10 mmol Tris (pH 8.0), 1 mmol EDTA]. Following an additional purification by phenol/chloroform/ispropanol alcohol (25∶24∶1) extraction and washing with 700 g·L^{-1} ethanol, the DNA pellet was vacuum-dried and re-dissolved in TE buffer. The concentration of DNA was measured using fluorometric assay. The template DNA was stored at 5 mg·L^{-1} for routine RAPD analysis.

1.3 DNA amplification

The PCR protocol was modified from Williams *et al*'smethod[11]. Random 10-nucleotide primers, viz. kit-R, kit-T as well as OPA-08, OPE-08, OPL-12, OPP-06, OPU-06 and OPW-15 from Operon Technologies (Alameda, Ca USA) were used, among which the last five primers and OPR06 were used for discriminating the interspecific hybrid clones and horticultural varieties of *Chamaecyparis obtusa* and *C. pisifera*[11]. Amplification reactions were performed in a total volume of 25 μL containing 10 mmol Tris-HCl (pH 8.5), 50 mmol KCl, 2 mmol $MgCl_2$, 0.01 g·L^{-1} gelatin, 100 μmol dNTP each, 0.5 units of *Taq* DNA polymerase (Perkin-Elmer Applied Biosystems), 25 ng of genomic template DNA, and 5 pico-moles of a random primer. PCR amplification was performed on a Perkin-Elmer DNA Thermal Cycler 9700 programmed at 94 ℃ for the preliminary 5 minutes followed by a total of 45 cycles of 1 minute at 94 ℃, 2 minutes at 36 ℃ and 2 minutes at 72 ℃, an extension of 7 minutes at 72 ℃ and a soak at 4 ℃. The RAPD products were resolved electrophoretically on 1.5% agarose gels incorporated with ethidium bromide [10 μL (10 g·L^{-1})·L^{-1}]. One control (blank) containing all the components of a typical PCR reaction but except the template DNA was tested in the preliminary screening of primers to confirm that amplification products represent amplified genomic DNA and not an artifact of the primer[7]. Sizes of the amplified products were estimated by using a Marker 4 DNA ladder (Wako, Nippon Gene). After running at 80V for about 5 h, the gel was photographed using the Multi-Analyst system (Bio-Rad Laboratories, CA 94957).

1.4 Genetic analysis of RAPD variants

In the genetic analysis, amplification of DNA was repeated at lest twice. Only the fragments whose presence and absence was reproducible and unambiguous were retained. Diffuse and/or very weak fragments were not scored because such fragments have been reported to possess the greatest propensity for poor reproducibility[12,13]. To study inheritance of the fragmems in the F_1 progeny, 15 individuals were analyzed for each cross. Based on the assumption that there is a diploid single locus mode of inbheritance, the agreement of the observed segregation ratios of RAPD fragments with those expected for a dominant Mendelian trait for each set of diallel crosses was calculated using the χ^2-tests.

2 Results

In the preliminary study, we tried to assess several genetic properties of RAPD fragments that are relevant to their use in the genetic analysis of *C. obtusa*. To identify primers that detect polymorphism, 46 primers were screened using DNA from needles of 5 parents shown in Table 1. Of these 46 random primers, 28 primers (OPA08, OPP08, OPR01, OPR03, OPR04, OPR05, OPR07, OPR08, OPR09, OPR11, OPR12, OPR015, OPR17, OPR18, OPR19, OPR20, OPT01, OPT05, OPT09, OPT10, OPT13, OPT14, OPT15, OPT16, OPTI9, OPT20, OPU06 and OPW15) failed to yield any amplification products. The remaining eighteen primers yielded 135 reproducible fragments and no amplified product was found with these primers in control. Of these 135 fragments, 42 fragments (31%) were polymorphic among the parents. The number of amplified polymorphic fragments varied from one to four per primer with an average of 2.1 fragments per primer. The size of fragments ranged from 290 to 1 100 bp. Only the fragments that showed polymorphism in at least one parent analyzed in this study were considered for the next genetic segregation analysis. Based on these preliminary results, we selected

seven primers (OPR02, OPT02, OPT04, OPT06, OPT07, OPT08 and OPT12) which gave the best amplification products and used them for the analysis of RAPD inheritance in 6 controlled crosses and their reciprocals.

Figure 1 shows the amplification by primer OPT07 in parents and progeny in the cross of Izu 3× Izu 5. The results from the present analysis of RAPD variation in parent and pooled progeny are summarized in Table 2. According to the banding patterns of each parent in preliminary amplification, each of 14 scorable and reproducible fragments amplified with 7 selected primers was identified by segregation of pooled progenies from several crosses. All the RAPD fragments occurring in parents over all the crosses were referred to 3 categories, in which a dominant character of the RAPD fragment variation was assumed.

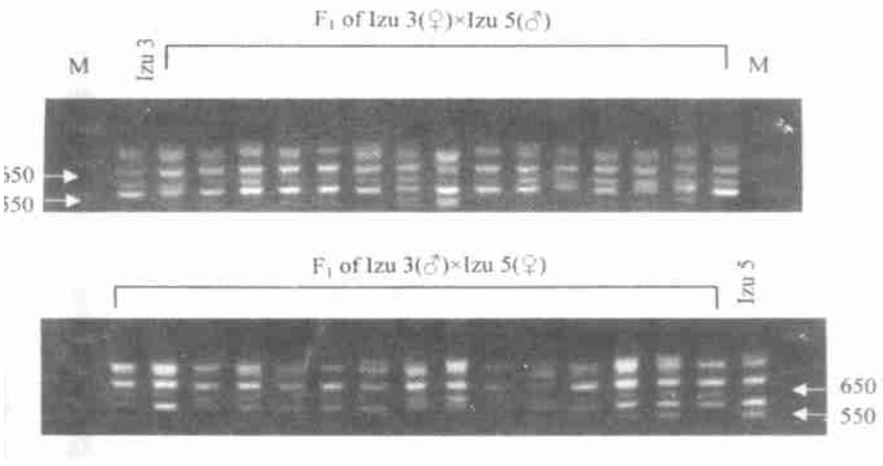

Figure 1 RAPD fragments amplified with the primer OPT07 in parents and progeny in the cross of Izu 3× Izu 5; M: DAN size marker (*Hae* III cut ΦX174)

The first category was those fragments shared by both parents. This was studied only for absent fragments observed in at least one of the four remaining parents. The absent fragment was found in one of the following three hypothetical combinations of the parental genotypes: AA× AA, AA× Aa or Aa× Aa. The first two combinations involving a dominant homozygote (AA) were expected to yield monomorphic progeny that shared the parental fragment. In the third cross between 2 dominant heterozygotes (Aa), the fragment was expected to segregate at 3: 1 among the progeny. We observed 19 cases where a fragment was shared by both parents, in eight of which the fragment occurred in all of the progeny. In the remaining 11 cases involving 7 fragments, each fragment was present in both parents but showed present/absent polymorphism in their progenies. In all these 11 cases, segregation of the fragment among the progenies did not deviate significantly ($P > 0.05$, χ^2-test) from the 3∶1 ratio expected for a dominant Mendelian trait.

The second category was that a fragment was present in one parent but absent in the other. This phenomenon occurs when one parent possessing a fragment is either a dominant homozygote (AA) or a dominant heterozygote (Aa) and the other a recessive homozygote (aa). In the former case (AA× aa), all the progeny were expected to possess the fragment found in one of the parents. In the latter case (Aa× aa), the fragment was expected to show present/absent polymorphism, and to segregate at a 1∶1 ratio among the progenies. We found 29 cases where a fragment was present in one parent but absent in the other. In 4 of these 29 cases, which were OPT04-1080 for Fuji 1× Fuji 6 and Izu 3× Fuji 1, OPT06-440 for Izu 5× Fuji 6 and OPT12-610 for Fuji 1× Fuji 6, the fragment occurred in all the full-sib progeny. In the remaining 25 cases involving 11 fragments, with the exception of OPT08-1100 for progeny of Izu 3× Fuji 1, the fragment present in one parent showed present/absent polymorphism in the progenies, which did not deviate significantly ($P > 0.05$, χ^2-test) from the 1∶1 ratio expected for a dominant Mendelian trait.

Table 2 Segregation of RAPD fragments for controlled crosses and the parental genotypes inferred from sets of controlled crosses

Fragment[1]	Cross	Parene's phenotype[2]		Progeny				$\chi^{2(3)}$	Inferred parent's genotype	
				Expected		Observed				
		P_1	P_2	+	−	+	−		P_1	P_2
OPR02-1080	Izu 3× Fuji 1	+	+	30	0	30	0		Aa	AA
OPR02-1080	Izu 3× Fuji 5	+	+	22.5	7.5	22	8	0.022	Aa	Aa
OPR02-1080	Izu 3× Fuji 6	+	+	22.5	7.5	21	9	0.200	Aa	Aa
OPR02-850	Izu 3× Fuji 1	+	−	15	15	17	13	0.533	Aa	aa
OPR02-850	Izu 3× Fuji 5	+	+	22.5	7.5	2.5	5	0.556	Aa	Aa
OPR02-850	Izu 3× Fuji 6	+	+	22.5	7.5	22	7	0.022	Aa	Aa
OPR02-800	Izu 3× Fuji 1	+	+	22.5	7.5	28	2	2.689	Aa	Aa
OPR02-800	Izu 3× Fuji 5	+	+	22.5	7.5	24	6	0.200	Aa	Aa
OPR02-800	Izu 3× Fuji 6	+	+	22.5	7.5	23	7	0.022	Aa	Aa
OPT02-1080	Izu 3× Fuji 1	−	+	15	15	17	13	0.533	aa	Aa
OPT02-1080	Izu 3× Izu 5	−	+	15	15	16	14	0.133	aa	Aa
OPT02-1080	Izu 5× Fuji 6	+	+	22.5	7.5	19	11	1.089	Aa	Aa
OPT04-1080	Fuji 1× Fuji 6	+	+	30	0	30	0		AA	Aa
OPT04-1080	Izu 3× Fuji 1	−	+	30	0	30	0		aa	AA
OPT04-1080	Izu 3× Fuji 6	−	+	15	15	16	14	0.133	aa	Aa
OPT06-770	Izu 3× Fuji 5	−	+	15	15	17	13	0.533	aa	Aa
OPT06-770	Izu 3× Izu 5	−	+	15	15	14	16	0.133	aa	Aa
OPT06-770	Izu 5× Fuji 6	+	−	15	15	13	17	0.533	Aa	aa
OPT07-650	Izu 3× Fuji 1	+	−	15	15	13	16	0.333	Aa	aa
OPT07-650	Izu 3× Izu 5	+	−	15	15	17	12	0.867	Aa	aa
OPT07-650	Izu 5× Fuji 6	−	+	15	15	19	11	2.133	aa	Aa
OPT07-550	Izu 3× Fuji 1	−	+	15	15	18	11	1.667	aa	Aa
OPT07-550	Izu 3× Izu 5	−	+	15	15	13	16	0.333	aa	Aa
OPT07-550	Izu 5× Fuji 6	+	+	30	0	30	0		Aa	AA
OPT08-1100	Fuji 1× Fuji 6	−	+	15	15	18	12	1.200	aa	Aa
OPT08-1100	Izu 3× Fuji 1	+	−	15	15	21	9	4.800*	Aa	aa
OPT08-1100	Izu 3× Fuji 5	+	−	15	15	15	15	0.000	Aa	aa
OPT08-1100	Izu 3× Fuji 6	+	+	22.5	7.5	24	6	0.200	Aa	Aa
OPT08-1100	Izu 3× Izu 5	+	+	30	0	30	0		Aa	AA
OPT08-1100	Izu 5× Fuji 6	+	+	30	0	30	0		AA	Aa
OPT08-550	Fuji 1× Fuji 6	+	−	15	15	15	15	0.000	Aa	aa
OPT08-550	Izu 3× Fuji 1	−	+	15	15	16	14	0.133	aa	Aa
OPT08-550	Izu 3× Fuji 5	−	+	15	15	13	17	0.533	aa	Aa
OPT08-550	Izu 3× Fuji 6	−	−	0	30	0	30		aa	aa
OPT08-550	Izu 3× Izu 5	−	+	15	15	14	16	0.133	aa	Aa
OPT08-550	Izu 5× Fuji 6	+	−	15	15	16	14	0.133	Aa	aa
OPT08-320	Fuji 1× Fuji 6	−	−	0	30	0	30		aa	aa
OPT08-320	Izu 3× Fuji 1	+	−	15	15	16	14	0.133	Aa	aa
OPT08-320	Izu 3× Fuji 5	+	+	30	0	30	0		Aa	AA
OPT08-320	Izu 3× Fuji 6	+	−	15	15	13	17	0.533	Aa	aa
OPT08-320	Izu 3× Izu 5	+	−	15	15	18	12	1.200	Aa	aa
OPT08-320	Izu 5× Fuji 6	−	−	0	30	0	30		aa	aa
OPT12-870	Fuji 1× Fuji 6	+	−	15	15	15	15	0.000	Aa	aa
OPT12-870	Izu 3× Fuji 1	+	+	22.5	7.5	21	9	0.200	Aa	Aa
OPT12-870	Izu 3× Fuji 6	+	−	15	15	16	14	0.133	Aa	aa
OPT12-610	Fuji 1× Fuji 6	+	+	30	0	30	0		Aa	AA
OPT12-610	Izu 3× Fuji 1	−	+	15	15	17	13	0.533	aa	Aa
OPT12-610	Izu 3× Fuji 6	−	+	30	0	30	0		aa	AA

Notes: (1) The fragments is expressed as primer number plus the fragment size. (2) +: fragment present; −: fragment absent. (3) The critical χ^2 at $P = 0.05$, 1 *df*. is 3.84. Values marked by an asterisk indicate a significant deviation from expected values

The third category is the fragment absent from both parents. This was studied only for fragments observed in at least one of the remaining 4 parents. Two fragments, viz. OPT08-320 and OPT08-550, were tested for their segragation. The fragment OPT08-320 was absent in parents such as Fuji 1, Fuji 6 and Izu 5, and OPT08-550 was absent in parents such as Izu 3 and Fuji 6. As expected for a cross between two recessive homozygotes, fragment OPT08-320 was absent from pooled progenies of Fuji 1× Fuji 6 and Izu 5× Fuji 6, and OPT08-550 was absent from progenies of Izu 3× Fuji 6. The fragments OPT08-550 and OPT08-320 were also tested in terms of present fragments of the cross. The segregation of these fragments in the progeny from the parents with these fragments either did not deviate significantly from the expected 1∶1 ratio or yielded monomorphic progeny that shared the parental fragment. Most early studies concerned with RAPD inheritance in controlled crosses reported results only for parents and progenies with a fragment. However, assuming the dominant character of the RAPD polymorphism it appears necessary to test the case where both parents show fragment absence, i. e., both parents are recessive homozygotes. In addition to the analysis of parents and progenies that showed the presence of a fragment, the analysis of parental genotypes with some fragment absent also provides relevant information about the genetic behavior of RAPDs.

In summary, the segregation of all 14 variable fragments found in this study complies with expected Mendelian inheritance and is consistent with the mode of a dominant single-locus trait. The identification of both the genetic control of these RAPD variants and the transmission mode of controlled genes from parents to progeny implies the potential for applying these fragments as genetic markers to further genetic research in *Chamaecyparis obtusa*.

3 Discussion

Among the primers used in the study, OPA08, OPE08, OPL12, OPP06, OPR06, OPU06 and OPW15 have discriminated *C. obtusa* from *C. pisifera*, and successfully identified their horticultural varieties[11]. Under the conditions of the present study, there were not any RAPD products amplified from primers OPA-08, OPP-08, OPU-06 and OPW-15 which amplified the species-specific bands for *C. obtusa*. Such an unclear reproducibility between laboratories and even between studies in the same laboratory is one of the major concerns in the use of RAPD analysis. In general, the RAPD technique utilizes low input substrate and many cycles of amplifcation. The fragments that are amplified during RAPD analysis probably represent the most successful products among many competitive candidates for amplification. Therefore, even minor changes in the reaction conditions may alter the amplifying results, and result in a specific fragment to be amplified in one genetic background but not in another. This is the artifactual variation characteristic of RAPD and increases the difficulty in the application of this technique.

Characteristics of dominant Mendelian traits of RAPD fragments and the possibility of applying these fragments as genetic markers have been confirmed based on the analysis of controlled crosses in *C. obtusa* of the present study (Table 2). A similar mode of inheritance of RAPD fragments has been reported in some other forest species[2, 5, 11, 13]. However, published data on RAPD inheritance based on the analysis of controlled crosses is only available in *Picea glauca*, *Pseudotsuga menziesii*[5] and *Pinus sylvestris*[13]. The present study tested each RAPD fragment for its reproducibility of segregation in at least three controlled crosses. Such a multiple cross design could eliminate the accidental effects of segregating deviations from the expected due to small progeny size[2, 5], confirm the parental genotypes from several crosses and infer the parental genotypes of the crosses in which the fragment was detected in both parents and in all the progeny. Our present results provide additional support for the usefulness of RAPD fragments as genetic markers.

In terms of application of the RAPD fragments, most of the earlier studies on RAPDs have focused on the use of this marker for mapping or genetic diagnostics[3, 5, 11, 15]. Moreover, RAPD is particularly useful when vegetative material is available in minute amounts and when tissues contain high levels of secondary metabolites that inhibit

enzyme activity. An example is *Betula alleghaniensis*, in which allozymes and RFLPs are of limited value[14]. We are more interested in applying the identified fragments as markers to estimation of genetic diversity fluctuation in reaforestation. The measurement of genetic diversity fluctuation in managed forests involves accurate measurement in a large number of organisms. Therefore, this demand to develop rapid and efficient techniques to measure polymorphisms. RAPD seems to be a right technique. However, the dominant character of RAPD markers implies that they would be less informative in population analysis than allozymes[14]. In conifers, this disadvantage can be alleviated by using haploid macrogametophytes of the individuals investigated[16]. Such an approach requires the analysis of multiple samples per individual which increases the amount of work substantially, and is limited to only seed-producible and seed-analyzable individuals. In *Chamaecyparis obtusa*, thousand-kernel weight of seeds with big a pterygium varies from 1.3 to 3.2 g[17] so that it is not easy to analyze the macrogametophytes. We discriminated dominant heterozygotes from homozygotes by testing progenies of many crosses involving one of the parents with other panents that do not exhibit these specific fragments. These parental genotypes could also be inferred even without analyzing macrogeametophytes. However, the establishment of diallel or partial diallel crosses is an indispensable prerequisite for such a multiple cross analysis and it is too laborious to be practically applied in general. In the other study recently made by us, we used simpler indices (e.g. Jaccard's coefficient and Shannon's information measure) computed directly from RAPD phenotype frequencies instead of the expected heterozygosity that is estimated from allelic frequency by using these identified RAPD markers to evaluate the changes of genetic diversity among developing seedlings in *C. obtusa*. The results were concordant with those of a previous study with the same materials using allozyme markers[18]. It seems that the limitations of RAPDs in dominance can be compensated by large numbers of easily accessible polymorphic markers that provide insights into many unexplored regions of the genome for detection of genetic diversity fluctuation in *C. obtusa* reaforestation.

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日本扁柏部分双列杂交试验的 RAPD 遗传分析

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摘要: RAPD 作为一简易的 DNA 分子标记, 在得以广泛应用的同时, 存在着随机扩增的 DNA 条带并不遵行显性遗传模式, 以及受 PCR 反应条件影响大等现象。以 5 个日本扁柏 *Chamaecyparis obtusa* 优树无性系及其部分双列杂交的 12 组合 (各含 30 个体) F_1 代为材料, 探讨 RAPD 标记的子代遗传及分离特征。研究结果表明: 优树无性系中供试的 46 个引物中, 14 个引物扩增了 42 条多态的片段, 筛选了其中 7 个引物扩增得来的 14 条片段进行分析, 在子代中均能找到其对应的片段, 且这些片段符合孟德尔的遗传分离规律。说明 RAPD 可作为遗传标记在日本扁柏中用于遗传分析。图 1 表 2 参 18

关键词: 日本扁柏; 部分双列杂交; RAPD; 分离分析