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SHORT COMMUNICATION

USING SSR MARKER TO IDENTIFY ALLELE VARIATION OF SOMACLONAL MUTANTS IN INDICA RICE

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ABSTRACT

Using microsatellite markers to identify allele variation of somaclonal mutant to detect mutants from callus in tissue culture and expected the mutant ratios of allele in this population. Tissue culture technique can detect mutants from somatic embryos. The changing of phenotypic mutant lines of OM1490 was not different from each other on statistical signification. On the contrary, genotypic mutants were significantly changed about DNA sequence under using SSR marker due to PCR technique to amplify simple sequence motif repeats, which were separated on PGE gel.

The allelic variation of OM1490 mutants were computed at 19.53% as compared to their parents. Among of mutants, line 19 was the most different from its parents and 30.9% alleles changed through tissue culture.

Key words: allele, indica rice, microsatellite, PGE gel, somaclonal variation

INTRODUCTION

Microsatellites or simple sequence repeats (SSRs) are simple sequence of tandemly repeats which can presently be a short motif of di-nucleotides, or tri-nucleotides, or tetra-nucleotides repeated and contains in 1-6 base pairs (bp) in length (Li et al. 2004). On the chromosome, SSRs occur once in every 18.8 kb, on the average, with one SSR per 23.8 kb and 16 kb on short and long arms, respectively. In genetic engineering, SSRs have played an important role in genome evolution (Tauz et al. 1986; Kashi et al. 1997; Toth et al. 2000). SSRs have become a popular type of co-dominant molecular marker in genetic analysis and plant breeding application (Yang et al. 1994; Olufowote al. 1997; Cho et al. 2000) and also been useful in integrating genetic, physical, and sequence-based maps of rice, and provided breeders and geneticists with efficient tool to link phenotypic and genotypic variations (Wu et and Tansley 1993; Akagi et al. 1996; Panaud et al. 1996; Temnykh et al. 2001. Therefore, they represent new sources of informative genetic markers (Weber et al. 1993).

Regeneration of plant from tissue culture often causes genetic change due to tissue severe stress exposed during plant regeneration (Hyun-Soo et al. 2004), so simple sequence repeats of few base pairs in length can find polymorphism of DNA from mutant populations. The type of DNA polymorphism could be detected only after polymerase chain reaction (PCR), amplification of DNA and separation on poly-acrylamide gel electrophoresis (Wu et el. 1993)

To identify whether microsatellites are suitable as a tool of marker-assisted selection (MAS) in somaclonal mutant population. In this paper, we report initial results regarding microsatellites in somaclonal mutants of indica rice variety that is difficult to get green plant regeneration to detect mutants from callus in tissue culture and expected the mutant ratios of allele in this population.

MATERIALS AND METHODS

Plant materials

Forty five somaclonal mutant lines in M_2 of OM1490 which is an indica cultivar derived from OM606 / IR44592-62-1-3-3. Mature embryos of this cultivar were cultured on MS medium containing 1 ppm 2, 4-D and 0.2 ppm NAA for callus induction . Green plant

regeneration was done on MS without plant regulator agent and grown in net house for self-pollination to obtain M_2 mutant lines for analysis. In this study, we also collect five wild types' samples together with mutants for analyzing.

DNA isolation

Seedlings of 50 lines at 10 day-old in germination room were used for DNA extracting. Genomic DNAs were extracted by SDS mini-scale method, 5% of Tris (pH 8.0) 50mM, 5% EDTA (pH 8.0) 25mM, 6% of NaCl 3000mM, 10% of SDS 1% and 74% of sterilized de-ionized water in v/v volume. Total extracted DNA was dissolved in 50µl TE and used 1µl for PCR reaction.

SSR markers and PCR applying

A total of 20 SSR markers were used in this study. All of primers used have annealing temperature at 55^0C and expected PCR size product with 128.25 bp in average with PCR programme (cycle 1: 94^0C for 2 minutes, cycle 2: 94^0C for 45 seconds, cycle 3: 55^0C for 45 seconds, cycle 4: 72^0C for 1 minute, cycle 5: go to cycle 2 with 29 times, cycle 6: 72^0C for 8 minutes, and store at 4^0C)

Data analysis

Diversity level of SSR loci was evaluated with genetic diversity index ($1-\sum p^2_i$), where p_i is the frequency of the i^{th} allele of the locus. For subdivided sample, the diversity of the total sample can be partitioned into component due to the differentiation among and diversity of within sub-samples (Nei 1973). Statistical programme, NTSYSpc 4.0 was used to analyze the data.

RESULTS AND DISCUSSION

Survey of polymorphic ratio per loci

When testing with twenty SSR loci (table 1), four of them have no polymorphism in the population. The average of pholymorphic rate is 49.85% in every loci of SSR markers. On the other hand, total of alleles getting from twenty primers is 85 alleles. In every locus is about 4 alleles in average of all individuals in this experiment. In addition, all the SSR primers selected were showed polymorphism in mutant population with primer RM240 at the rate of 81.30%. Of 20 primers, 16 SSRs exhibited polymorphism which showed that the changing of molecular construction in the population at high rate.

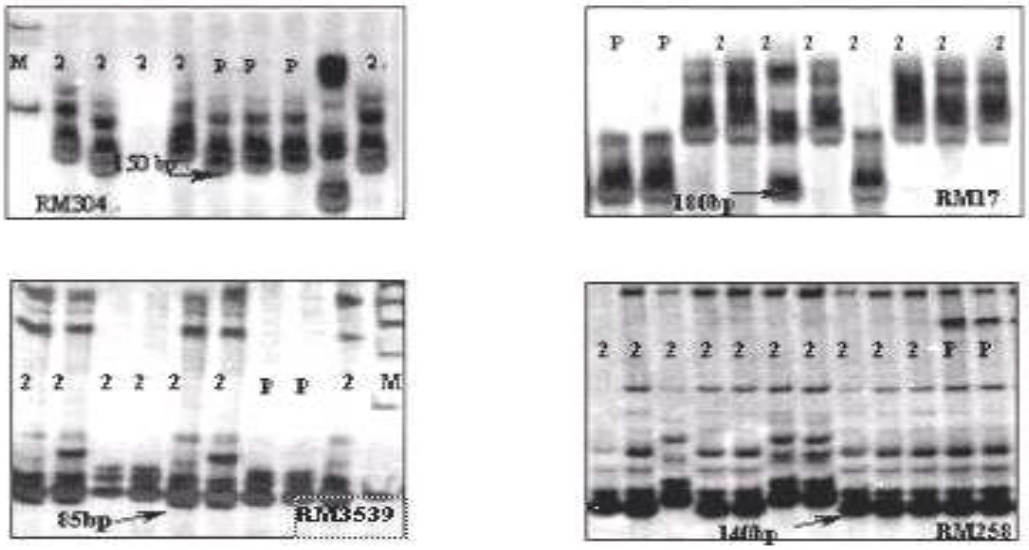
Table 1. Number of allele and percentage of polymorphic individuals per locus

Locus	No. of alelle	% Psm*	Locus	No. of alelle	% Psm*
RM254	6	26.84	RM151	4	62.50
RM19	4	29.89	RM273	3	66.67
RM304	5	34.36	RM17	3	67.35
RM1195	5	38.40	RM240	3	81.30
RM106	4	40.00	RM35	2	-
RM29	9	42.08	RM297	3	-
RM127	4	44.50	RM309	3	-
RM253	2	48.97	RM107	3	-
RM5359	6	55.21	RM278	3	-
RM258	9	59.78	RM267	4	-
Mean	4.25	49.85			
Max	9.00	81.30			
Min	2.00	26.84			
Total	85.00				

- :Not polymorphism; *Polymorphism

Evaluating variation of alleles

In the population including soma clones which is from wild OM1490, the changing of allele among mutants and their wild genotype was calculated at 19.53% allele (table 2). In addition, variation among lines was high, maximized and minimized changing of allele was up to 30.9 % and 7.7%, respectively. So that, the mutant at M_2 of this material from somaclonal mutants created many novel alleles under using SSR markers that occurred in genetic sequence construction (Figure 1).



2: M_2 mutants; P: parents; M: ladder

Figure 1: Typical pophymorphic loci on PAGE with PCR products using SSR primers

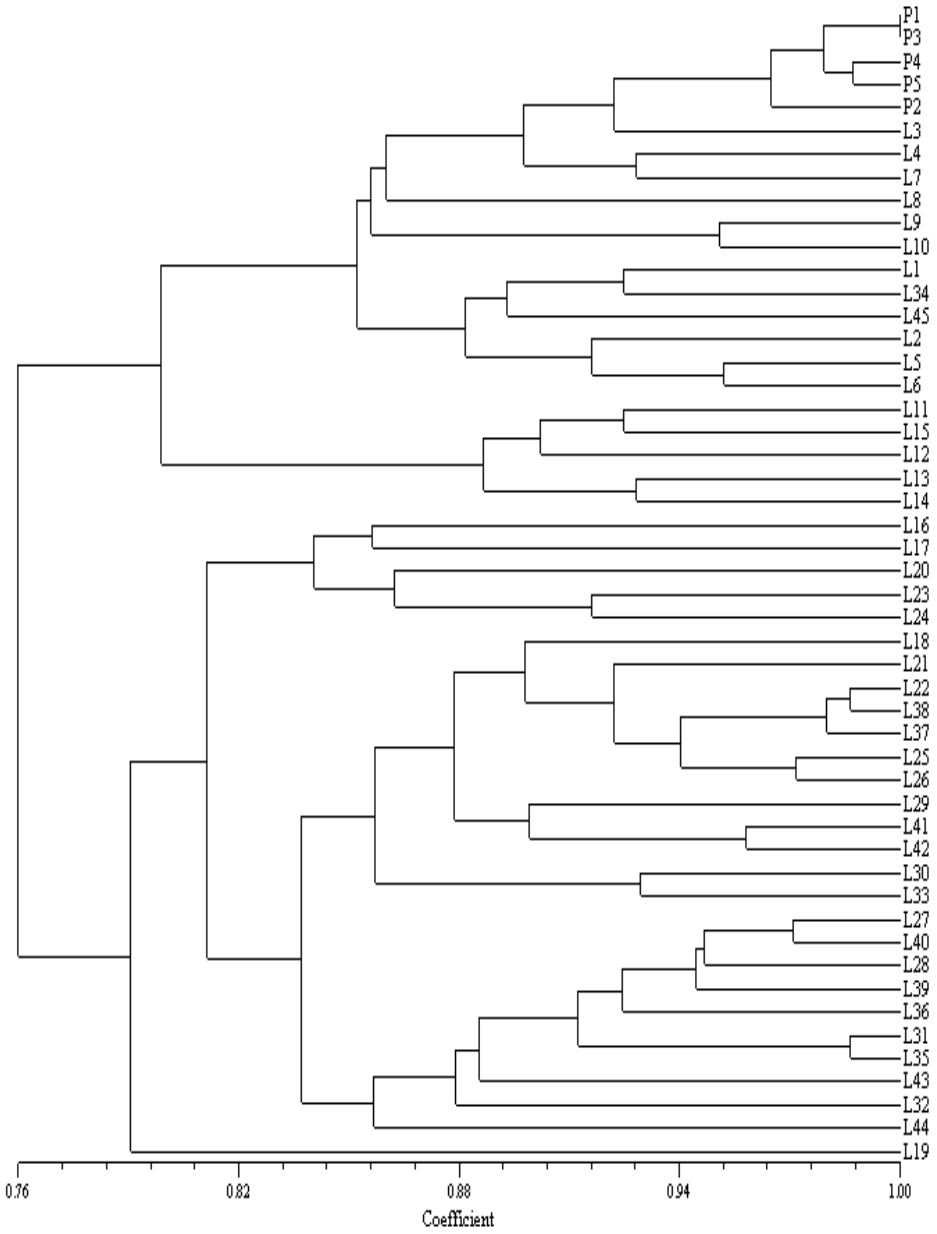
Table 2: Changing of allele between mutants and parents

No	%*	No	%*	No	%*
L1	13.10	L16	17.30	L31	14.30
L2	9.90	L17	23.70	L32	26.60
L3	7.70	L18	24.50	L33	22.60
L4	10.60	L26	23.50	L34	14.80
L5	13.30	L20	26.50	L35	13.90
L6	9.40	L21	25.50	L36	22.50
L7	8.90	L22	26.00	L37	24.50
L8	14.30	L23	20.80	L38	23.10
L9	14.40	L24	23.50	L39	21.20
L10	11.60	L25	26.20	L40	21.50
L11	19.20	L19	30.90	L41	29.90
L12	14.80	L27	24.10	L42	28.60
L13	19.70	L28	17.40	L43	26.50
L14	19.40	L29	20.00	L44	21.20
L15	14.80	L30	19.40	L45	17.20
Max					30.90
Min					7.70
Mean					19.53

*: % allele in different between mutant and parents

The relationship between mutants and their papents

The above evaluation was changing about 20% allele as compared to parents, phylogenetics which exhibited in figure 2, indicated high diversity of mutants (figure 1 and 2). Many clusters were classified, but the mutants closely correlated to their parents at least at 0.76 coefficient. On the other hand, the coefficient at 0.82 devided the population in to 5 clusters. One of five was line L19 which was obviously separated form the others. The parent lines were classified into in the same cluster.



P: parents lines, L: Mutant lines

Figure 2. Phylogenetic relationship between parent and their mutant lines

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Sử dụng SSR marker để xác định sự biến dị dòng đột biến soma

SSR đã được sử dụng để nghiên cứu sự biến dị của các dòng soma phát triển từ mô sẹo của OM1490, được nuôi cấy trong môi trường MS (1 ppm 2, 4-D và 0.2 ppm NAA) để kích thích mô sẹo. Sự thay đổi kiểu hình của các dòng đột biến tế bào soma và dòng gốc không khác nhau có ý nghĩa. Nhưng sự khác biệt thông qua đánh giá kiểu gen với sự trợ giúp của SSR cho thấy rất rõ ràng với 5 cluster về di truyền được phân lập. Biến dị của mutant với giá trị 19,53% được ghi nhận so với dòng gốc OM1490.