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MITE-AFLP를 이용한 자포니카 벼의 다양성 검정

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MITE-transposon Display in Japonica Rice

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Objective

Miniature inverted transposable elements (MITEs) are abundant genomic components in plant including rice. MITE-transposon display (MITE-TD) is an Amplified fragment length polymorphism (AFLP)-related technique based on MITE sequence. In this study, we used the MITE-AFLP for genetic diversity and relationship of the 114 *japonica* species. Of the several MITEs, using the *mPing* family was applied to detect polymorphism based on PCR amplification. In MITE-TD, the *Bfa*I adaptor primer and the specific primer derived from *mPing* terminal inverted repeat (TIR) region were used to PCR amplification. This study was conducted to discriminate among the 114 Japonica rice using AFLP technique and the relationship of the 114 species based on similarity coefficient were compared and candidated primer was used to applied to construction of a molecular linkage map using Milyang23 and Hapcheonaengmi3 Recombinant Inbred Lines

Materials and methods

○ Plant materials :114 *Oryza sativa* japonica, 80 BC1F7 : Milyang 23 and Hapcheonaengmi 3 Recombinant Inbred Lines

○ AFLP-MITE : Genomic DNA extraction, Enzyme digestion, Ligation, 1st and 2nd PCR, Comparision of DNA polymorphism among materials, Identification of *Japonica* species

○ Data analysis :NTSYS (Numerical Taxonomy and Multi Analysis System)-pc (version2.1), Map maker software (version2.0)

Results

1. Genetic dirversity of 114 *japonica* species evaluated using 9 MITE-AFLP primers and generated a total 169 polymorpic bands. The PIC values arranged from 0. 293 to 0.499 with an average 0.363.
2. Two primers of 9 primer combination, *Bfa*I+G and *Bfa*I+C, were able to the identification among the 114 *japonica* species.
3. Analysis of the 80 RILs with MITE-AFLP identified a total of 31 polymorpic bands and 5 MITE-AFLP markers were integrated into the molecular linkage map. MITE-AFLP markers were distributed throughout the four chromosomes. These genetic loci were located on chromosome 1, 4, 7 and 9 respectively.

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Table 1. MITE-AFLP Adaptors and Primers used in this study and PIC value of polymorphism

		Sequence	No. of polymorphic bands	PIC average
Adaptors				
		5'-GACGATGAGTCCTGAG-3'		
		3'-TACTCAGGACTCAT-5'		
Primers				
	*Bfa*I + 0	5'-GACGATGAGTCCTGAGTAG-3'		
	*Bfa*I + A	5'-GACGATGAGTCCTGAGTAGA-3'	17	0.411
	*Bfa*I + T	5'-GACGATGAGTCCTGAGTAGT-3'	16	0.426
	*Bfa*I + G	5'-GACGATGAGTCCTGAGTAGG-3'	15	0.382
	*Bfa*I + C	5'-GACGATGAGTCCTGAGTAGC-3'	24	0.411
	*Bfa*I + AT	5'-GACGATGAGTCCTGAGTAGAT-3'	16	0.422
	*Bfa*I + AC	5'-GACGATGAGTCCTGAGTAGAC-3'	29	0.295
	*Bfa*I + TG	5'-GACGATGAGTCCTGAGTAGTG-3'	23	0.337
	*Bfa*I + AGC	5'-GACGATGAGTCCTGAGTAGAGC-3'	15	0.317
	*Bfa*I + ACT	5'-GACGATGAGTCCTGAGTAG ACT -3'	14	0.269

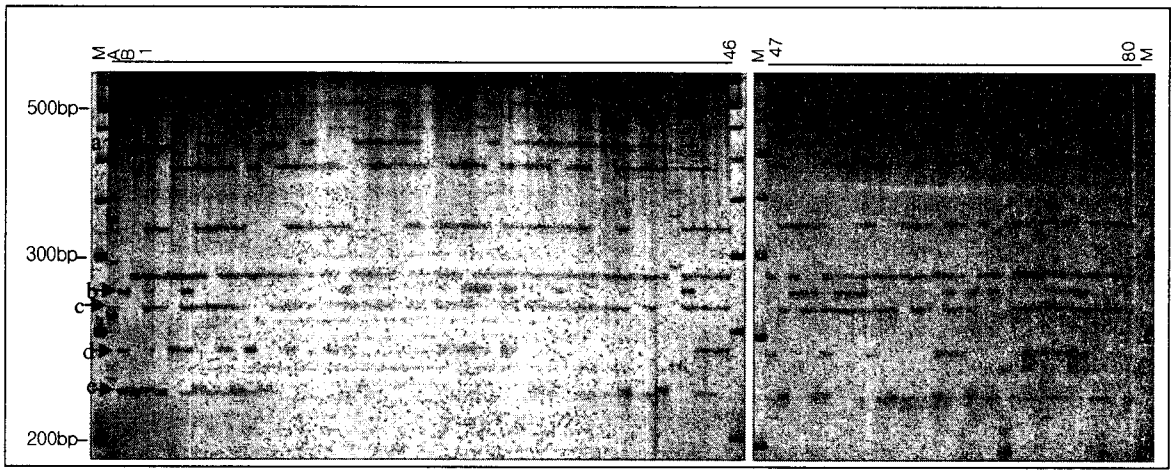

Fig. 1. Polymorphism of MITE-AFLP. The primer was *Bfa*I+0base. Arrows indicate specific marker for a molecular linkage map construction. a, M445. b, M275. c, M265. d, M240, e, M225. Lane A, Milyang 23. Lane B, Hapcheonaengmi 3. Lane 1 ~ 80, BC1F7 backcross inbred lines. M, 50bp DNA ladder.