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SSR MARKERS AND THE APPLICATION TO CULTIVAR PROFILING IN PEACH

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Abstract

A total of 36 SSR markers were successfully developed in peach (*Prunus persica* L.) by using an enriched genomic and fruit cDNA libraries. Twenty-four SSR markers were developed from 60 sequences containing (AG)/(TC) repeat screened with the magnet beads method. Twelve cDNA clones containing microsatellite repeats were obtained from about 800 expressed sequence tag (EST) sequences from peach fruits. Parentage of 16 Japanese peach cultivars, including 9 cultivars bred by controlled hybridization, 2 from bud sport mutations and 5 from chance seedlings, was analyzed using 17 SSR markers. Parentage of all crossbreeding cultivars and pedigree of 4 cultivars originated from chance seedlings were confirmed because of transmission of SSR alleles without discrepancy. SSR analysis revealed that a bud sport cultivar 'Hikawa Hakuhou' was not a bud sport mutant of 'Hakuhou'. Although almost all peaches grown in Japan were originated from 'Hakutou', its genetic origin has not yet been identified. Genetic relationships between 'Hakutou' and other peaches with different origins were analyzed using SSR markers. 'Hakutou' showed a close relationship to 'Shanhai Suimitsutou' which was introduced from China about 150 years ago and that there is very high possibility of parent-offspring relationship between them.

DEVELOPMENT OF SSR MARKERS IN PEACH

1. Twenty-four and 12 SSR markers (simple sequence repeat, also designated as microsatellite) were developed in peach (*Prunus persica* L.) by using an enriched genomic and fruit cDNA libraries, respectively (Table 1).

2. A genomic library enriched for (AG)/(TC) sequences was constructed from the peach variety 'Akatsuki' using the magnet beads method. In this study, 114 positive clones were screened from 1,115 colonies. After sequencing the positive clones, we obtained 60 independent sequences containing 8 to 36 microsatellite repeats of (AG)/(TC) ca. 21 on the average. Out of the 60 sequences, 55 contained complete (AG)/(TC) repeats and the others had interrupted repeats or combined motifs of (AG)/(TC) and other units. Forty primer pairs were designed, and 24 of them could successfully amplify the target fragments. Twenty-two SSR loci showed polymorphisms in peach with 3-9 alleles per locus. Two microsatellites showed monomorphic patterns. Values of observed heterozygosity of the 24 loci ranged from 0 to 0.64, with an average of 0.15. Expected heterozygosity ranged from 0 to 0.87, with an average of 0.68.

3. Twelve cDNA clones containing microsatellite repeats were obtained from ca. 800 expressed sequence tag (EST) sequences, in which ca. 700 and ca. 100 clones were from young peach fruits of 25 days after flowering and from mature fruits of 110 days after flowering, respectively. Seven, 2, 2 and 1 cDNA clones included microsatellite repeats in the putative 5'-untranscribed region (UTR), 3'-UTR, transcribed region (TR) and in an unknown region. Several kinds of motifs were observed. Four loci contained microsatellite repeats of

3-6 bp units such as CGTCAT (M13b), CTCAT (M2b), CAA (M5a) and AAG (M11c). The remaining loci had complete or interrupted motifs of AG or TC repeats. Eight sequences showed significant homology to the registered genes in a database. Seven loci showed polymorphisms in peach with 2-7 alleles per locus, whereas the other 5 loci exhibited monomorphic pattern. Values of observed heterozygosity of the 12 loci ranged from 0 to 0.29, with an average of 0.11. Expected heterozygosity ranged from 0 to 0.85, with an average of 0.38.

Parentage Analysis in Japanese Peaches

4. Parentage of 16 Japanese peach cultivars, including 9 cultivars bred by controlled hybridization, 2 from bud sport mutations and 5 from chance seedlings, was analyzed using 17 SSR markers (Table 2). The parent-offspring relationships of 9 crossbreeding cultivars were confirmed because SSR alleles were transmitted to offspring cultivars from their parents without discrepancy at all the used loci. 'Akatsuki' and 'Gyousei' showed identical SSR genotypes, which suggested that 'Gyousei' was derived from a bud sport mutation. In contrast, another bud sport cultivar, 'Hikawa Hakuhou', showed different SSR genotypes from those of the original cultivar 'Hakuhou' at 12 SSR loci, indicating that 'Hikawa Hakuhou' was not a bud sport mutant of 'Hakuhou'.

5. Four cultivars that presumably originated from chance seedlings, i.e., 'Abe Hakutou', 'Kawanakajima Hakutou', 'Kouyou Hakutou' and 'Shimizu Hakutou', shared one allele with their putative parent 'Hakutou' at each SSR locus. SSR analysis suggested that these 4 cultivars were not bud sport mutants, but offsprings of 'Hakutou'. It was concluded that SSR markers could be effectively utilized for parentage analysis in Japanese cultivated peaches.

'Shanhai Suimitsutou', the Origin of Japanese Peach Cultivars

6. Almost all peaches grown in Japan supposedly originated from 'Hakutou' by crossbreeding, so that they are closely related to it. For example, 'Hakuhou' and 'Akatsuki', offsprings of 'Hakutou', were obtained by controlled hybridization. There is a possibility that a lot of cultivars are chance seedlings or bud sports of 'Hakutou'. However, the genetic origin of 'Hakutou' has not yet been identified. In this study, genetic relationships between 'Hakutou' and other peaches with different origins were analyzed, using 10 SSR markers.

7. SSR analysis indicates that 'Hakutou' showed a close relationship to 'Shanhai Suimitsutou' which was introduced from China about 150 years ago and that there is a possible parent-offspring relationship between them. Contrarily, another candidate cultivar 'Kintou' showed a close relationship to 'Hakutou' but not that of a parent-offspring because of a discrepancy of genotypes for the SSR loci. Parentage analysis of 'Hakutou' and 'Shanhai Suimitsutou' was conducted by 43 SSR loci, revealed that all SSR alleles were inherited by 'Hakutou' without any discrepancy from the putative parent 'Shanhai Suimitsutou'. These results indicate the very high possibility that 'Shanhai Suimitsutou' is a parent of 'Hakutou' and one of the original germplasm of the Japanese peaches.

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Table 1. Characteristics of 36 SSR markers developed in peach.
Motifs and PCR product size refer to sequenced alleles. T_a, annealing temperature; H_O and H_E denote observed and expected heterozygosities, respectively.

Locus	Motif	Source	PCR product size (bp)	T_a (°C)	No. of putative alleles	H_O	H_E
MA004b	$(GA)_{13}$	genomic DNA	86	50	6	0.07	0.80
MA005c	$(GA)_{15}$	genomic DNA	175	55	3	0.07	0.49
MA006b	$(AG)_{18}$	genomic DNA	295	55	6	0.14	0.70
MA007a	$(TC)_4C(CT)_{27}$	genomic DNA	132	55	6	0.14	0.78
MA009b	$(GA)_{16}$	genomic DNA	132	55	3	0.29	0.47
MA010a	$(GA)_{20}$	genomic DNA	122	55	6	0.14	0.78
MA013a	$(AG)_{25}$	genomic DNA	211	55	6	0.14	0.80
MA014a	$(GA)_{21}$	genomic DNA	165	55	5	0.07	0.71
MA015a	$(AG)_{21}$	genomic DNA	177	52	7	0.21	0.81
MA016b	$(GGA)_3GAA(GGA)_3$	genomic DNA	103	55	1	0.00	0.00
MA017a	$(GA)_7CG(GA)_{19}$	genomic DNA	164	55	8	0.64	0.85
MA019a	$(GA)_{16}$	genomic DNA	108	55	4	0.00	0.71
MA020a	$(AG)_{23}$	genomic DNA	180	55	6	0.21	0.74
MA021a	$(AG)_{21}$	genomic DNA	227	55	1	0.00	0.00
MA023a	$(AG)_{24}$	genomic DNA	192	55	7	0.21	0.79
MA024a	$(GA)_{20}$	genomic DNA	244	55	7	0.00	0.79
MA026a	$(GA)_{24}$	genomic DNA	191	55	6	0.14	0.76
MA027a	$(GA)_{28}$	genomic DNA	159	55	9	0.14	0.87
MA030a	$(GA)_{12}$	genomic DNA	237	55	4	0.00	0.63
MA031a	$(GA)_{16}$	genomic DNA	131	55	5	0.21	0.82
MA034a	$(AG)_{18}$	genomic DNA	220	55	7	0.29	0.67
MA035a	$(GA)_{19}G(GA)_2$	genomic DNA	177	55	7	0.14	0.83
MA039a	$(GA)_{23}$	genomic DNA	188	50	6	0.07	0.54
MA040a	$(GA)_{17}$	genomic DNA	222	55	7	0.21	0.86
M1a	$(CT)_{12}$	cDNA	80	55	4	0.21	0.49
M2b	$(CTCAT)_9$	cDNA	295	55	2	0.07	0.39
M3b	$(AG)_{13}G(GA)_6$	cDNA	224	55	3	0.14	0.66
M4c	$(TC)_{17}$	cDNA	78	55	6	0.29	0.66
M5a	$(CAA)_5$	cDNA	132	55	1	0.00	0.00
M6a	$(AG)_{14}$	cDNA	195	55	4	0.21	0.73
M7a	$(GA)_4GC(GA)_6$	cDNA	142	55	1	0.00	0.00
M9a	$(AG)_4(GC)_2(AG)_8$	cDNA	138	55	1	0.00	0.00
M11c	$(AAG)_7$	cDNA	101	55	1	0.00	0.00
M12a	$(AG)_{13}$	cDNA	176	55	7	0.14	0.85
M13b	$(CGTCAT)_4$	cDNA	182	55	1	0.00	0.00
M15a	$(CT)_{16}$	cDNA	135	55	5	0.21	0.78

Table 2. Peach varieties used for parentage analysis

Variety name	Breeding method	Described parentage	Results in this study
Akatsuki	crossbreeding	Hakutou x Hakuhou	Parentage confirmed
Yuuzora	crossbreeding	Hakutou x Akatsuki	Parentage confirmed
Saotome	crossbreeding	Hakuhou x Robin[a]	Parentage confirmed
Chiyohime	crossbreeding	Kouyou Hakutou x Saotome	Parentage confirmed
Yoshihime	crossbreeding	Akatsuki x 21-18	Parentage confirmed
Masahime	crossbreeding	Akatsuki x 21-18	Parentage confirmed
Akizora	crossbreeding	Nishino Hakutou[a] x Akatsuki	Parentage confirmed
Natsuotome	crossbreeding	Akatsuki x Yoshihime	Parentage confirmed
Hakuhou	crossbreeding	Hakutou x Tachibana Wase[a]	Parentage confirmed
Gyousei	bud sport mutation	Bud sport of Akatsuki	Bud sport confirmed
Hikawa Hakuhou	bud sport mutation	Bud sport of Hakuhou	Not a bud sport of Hakuhou
Abe Hakutou	chance seedling	Hakutou x Ookubo	No discrepancy in parentage
Kawanakajima Hakutou	chance seedling	Shanhai[a] x Hakutou	No discrepancy of Hakutou as the parent
Kouyou Hakutou	chance seedling	Hakutou x ? or bud sport of Hakutou	No discrepancy of Hakutou as the parent, not a bud sport of Hakutou
Shimizu Hakutou	chance seedling	Hakutou x ?	No discrepancy of Hakutou as the parent
Ookubo	chance seedling	Hakutou x ?	Discrepancy at MA023 locus

[a]not used in this study

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