INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2000/02–3–187–191

Genomic DNA Variation associated with Phase Change in Crab Apple and Peach

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ABSTRACT

Random Amplified Polymorphic DNA (RAPD) techniques were used to detect genomic variation occurred in transition from juvenile to adult state of Malus micromalus, Malus hupehensis (crab-apple), Prunus persica (peach) seedling trees. One hundred primers of oligonucleotides decemer in case of Malus species and 40 for peach were tested. More than 75 % primers amplified clear-bright bands. Only primer S216 and S219 revealed differences between amplified DANs of two phases from Malus micromalus and Malus hupehensis respectively. Two primers S216 and S257 generated DNA polymorphic patterns between juvenile and adult phase of peach indicating that differential amplification might have taken place during phase change.

Key Words: Genomic variation; RAPD; Phase; *Malus micromalus*; *Malus hupehensis*; *Prunus persica*

INTRODUCTION

Several morphological and physiological changes are associated with transition from juvenile to sexually competent, mature state of plants. These depend upon species to species, and include leaf shape, phylotaxy, anthocyanin pigmentation, thorniness, shoot growth vigor, apical dominance and rooting ability (Zimmerman *et al*., 1985). Changes occur in terminal meristems and to be carried over to the lateral meristems when these are formed. It is believed to involve stable changes in apical meristems that are transmitted through mitotic cell division, and expressed in the tissues, buds and cuttings that subsequently drive from them (Poethig, 1990; Nagl, 1990; Jin, 1993).

Millikan and Ghosh (1970) noted changes in nucleic acids associated with maturation and senescence in *Hedera helix*. Difference in mitochondrial DNA between adult and juvenile coastal red wood *Sequoia sempervirens* by restriction fragment analysis has been reported (Huang *et al.,* 1995). Walbot and Cullis (1985) have proposed rapid genomic changes involving chromosome rearrangement, chromosome imprinting gene deletion, amplification, and transposable elements within the lifetime of an organism. In plants, such phenomena as nuclear DNA alteration, endoreplication, DNA amplification, DNA rearrangement are found to occur during cell differentiation and plant development (Kraszewska *et al*., 1985; Kowles *et al*., 1990; Shang & Wang, 1991). This study reports genomic variation in relation to transition from juvenile to adult phase of crab apple and peach.

MATERIALS AND METHODS

Mature seedling trees of apple **Malus micromalus* (Makino), *Malus hupehensis* and peach *Prunus persica* grown in Zhejiang Agricultural University were used as study material. Young leaves with Juvenile characters from basal shoots < 50 cm height from ground level and young leaves with mature phase characters were collected from crown shoots of the same tree. Samples were washed thoroughly with tap water and rinsed with distilled water to remove insect eggs or any residual pesticide, dried with blotting paper and weighed 2 grams.

DNA extraction. Genomic DNA was extracted according to the method described by Chen *et al*. (1997). Briefly, plant material was ground to a fine powder in liquid nitrogen with the help of porcelain pestle and mortar and transferred to 50 mL polyvinyl tube and added 10 ml of solution I containing 0.4 mol/L glucose 3% soluble PVP, 2.3 % DDTC, 10 mM β- mercapto-ethanol, 20 mM pH 8 EDTA. To remove the cytoplasmic contents centrifuged at 10,000 rpm for 10 minutes at 4^{o}C and discarded the supernatant. Repeated the above step again. Added 10 ml of pre-warmed lysis solution II consisting of 100 mM Tris HCl pH 8, 20 mM EDTA 0.5 M NaCl and 1.5% SDS, and mixed with sample and incubated at 65^{o}C for 1 h. with occasionally gentle mixing, after addition of equal volume chloroform: ethanol: isopentanol 80:16:4, centrifugation was performed at 10,000 rpm for 10 minutes to separate the aqueous phase. Supernatant was transferred in to another tube and DNA was precipitated with equal volume of isopropanol and dissolved in TE buffer and subsequently purified twice with phenol and chloroform 1:1 and precipitated with 100% ethanol.

Malus Micromalus (Makino) originated in China,

found in wild form in north western and north central parts such as Beijing, Honan, Shandong and Hebei. It blooms profusely and have good vegetative growth. Due to these characters it is mostly used as an ornamental tree and as a rootstock for apple cultivars.

Hooked out with glass rod, washed with ethanol 70%, air dried and dissolved in TE buffer (pH 8) and quantified at OD_{260} and OD_{280} nm, by using spectrophotometer and also running on agarose gel along with standard λDNA, stained with ethidium bromide.

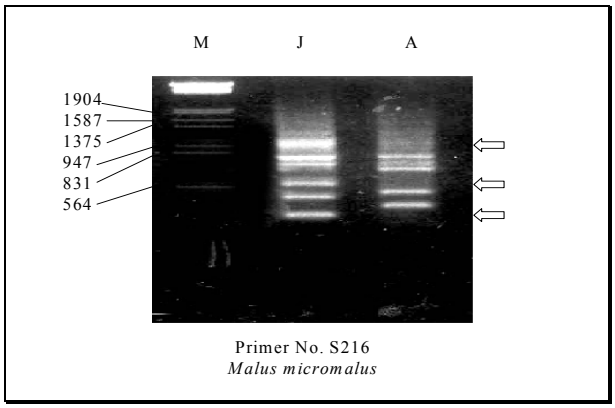
RAPD amplification. The template DNA (40 ng) was amplified in a total volume of 25 ul reaction mixture, containing 50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.1% Triton X-100, 2 mM $MgCl_2$, 0.2 mM of each d NTP, 2 uM of the primer, 1.25 u *Taq* DNA polymerase (Shanghai Promega). The mixture was overlaid with one drop of autoclaved mineral oil, transferred to a thermal controller (Model 1109-A2, China) preheated at 94°C. After 4 minutes at 94°C (DNA predenaturation), 45 thermal cycles were performed following the programme: 60s at 93°C (denaturation), 60s at 37°C (annealing), and 120s at 72°C (extension). Reactions were terminated by incubating 5 minutes at 72°C and stored the PCR products at 4°C untill used for electrophoresis. Amplified fragments were analysed along side with a standard λ DNA molecular markers cut by Hind III and ECOR I (Promega Madison, USA) on 1.7 % agarose gels (Bohenier) in 0.5x TBE, in the presence of ethidium bromide, and gels were photographed under UV light (Sambrook *et al.,* 1989). One hundred random primers of oligonucleotides decemer were obtained from Sangon co.Canda.

RESULTS AND DISCUSSION

Genomic DNA variation. Juvenile and mature phase leaf DNA of each seedling tree i.e. *Malus micromalus Malus hupeheneis*, and *Prunus persica* was used as substrate in RAPD reactions. Only bright and repeatable bands were considered and faints were ignored.

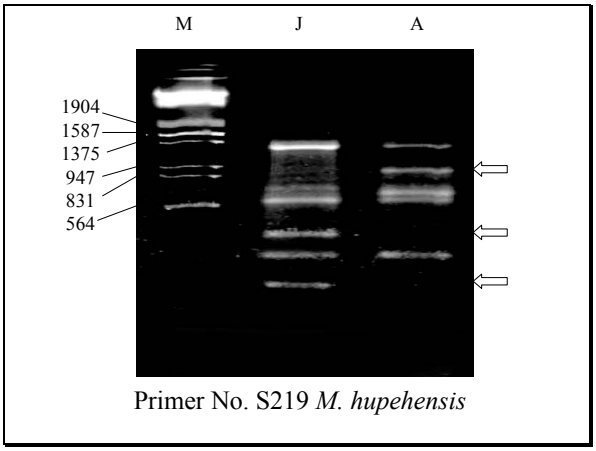
Malus micromalus. To find out the minor differences in comparison between Juvenile and Adult phase DNA of apple seedlings with genome containing 2n = 34 chromosomes, 100 random primers of oligonucleotides decemer were used. Seventy eight primers amplified 741 well-marked bands from DNA of both phases of the species (Table I). Primer S216 generated polymorphic pattern, distinguished by one fragment of approximately 400 units which was present only in mature phase and two fragments of about 300 and 200 base pair (bp) were only found in the amplified Juvenile DNA. Sequence of the primer is given in Table I, and representative gel pattern is shown in Fig. 1. The result was reproducible, even after one month the genomic DNA was taken out from stock solution, diluted to 40 μg/mL and in third replication, amplification product showed consistently potymorphic pattern, with an extra band of 700bp, distinctive in mature phase Fig. 1.

Fig. 1. RAPD-pattern amplified by primer S216 from two phases of *Malus micromalus*



Malus hupehensis. In the species of crab apple *Malus hupehensis*, genomic DNA from both phases were surveyed with same 100 primers to detect difference in nuleotides sequence. Seventy five primers produced 783 clear bands. Only one primer S219 revealed difference by one fragment of approximately 974bp, present only in mature phase and two fragments <500bp were characteristics of Juvenile phase Fig. 2.

Fig. 2. Polymorphic bands amplified by using primer S219 from J and A phase DNA of *Malus hupehensis*



Prunus persica. Peach seedling tree having flowers on just 45cm apical part of the crown-shoots was used as study material. Peach genome (diploid 2n = 16) is cooperatively smaller than that of crab apple. Forty

Table I. Numbers of RAPD products, polymorphic bands revealing differences in juvenile and adult phase leaf DNAs

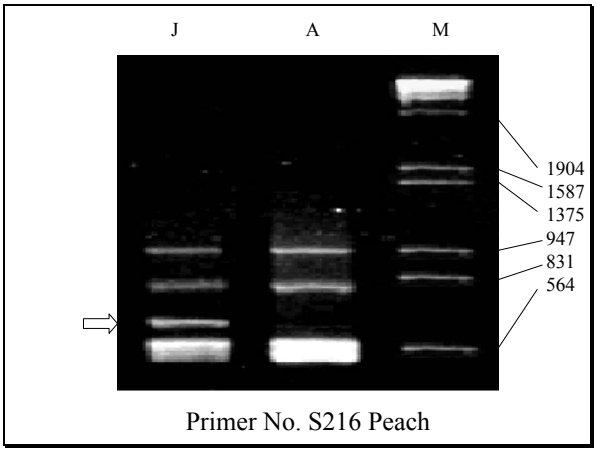
of crab apple and peach

Primer name	Sequences 5' to3'	M. micromalus		M. huphensis		Prunus persica	
		No. of total bands	Polymorphic bands	No. of total bands	Polymorphic bands	No. of total bands	Polymorphic bands
S201	GGGCCACTCA	5	-	3	-	6	-
S202	GGAGAGACTC	17	-	12	-	3	-
S203	TCCACTCCTG	13	-	10	-	3	-
S204	CACAGAGGGA	14	-	14	-	5	-
S205	GGGTTTGGCA	5	-	10	-	--	-
S206	CAAGGGCAGA	5	-	16	-	7	-
S207	GGCAGGCTGT	8	-	14	-	7	-
S208	AACGGCGACA	8	-	8	-	8	-
S209	CACCCCTGAG	10	-	10	-	6	-
S210	CCTTCGGAAG	0	-	0	-	2	-
S211	TTCCCCGCGA	15	-	8	-	3	-
S212	GGGTGTGTAG	8	-	16	-	5	-
S213	AGGACTGCCA	10	-	10	-	-	-
S214	AATGCCGCAG	5	-	10	-	-	-
S215	GGATGCCACT	10	-	18	-	-	-
S216	GGTGAACGCT	8, 6	3	15	-	5	1
S217	CCAACGTCGT	9	-	16	-	-	-
S218	GATGCCAGAC		-		-	-	-
S219	GTCCGTATGG	5	-	8	3	-	-
S220	GACCAATGCC	12	-	14	-	-	-
S221	TGACGCATGG	8	-	10	-	-	-
S222	AGTCACTCCC	3	-	14	-	-	-
S223	CTCCCTGCAA	1	-	8	-	-	-
S224	CCCCTCACGA	13	-	0	-	-	-
S225	TCCGAGAGGG	8	-	0	-	-	-
S226	ACGCCCAGGT	20	-	0	-	-	-
S227	GAAGCCAGCC	7	-	7	-	-	-
S228	GGACGGCGTT	10	-	10	-	-	-
S229	TGTACCCGTC	0	-	10	-	-	-
S230	GGACCTGCTG	5	-	3	-	-	-
S232	ACCCCCCACT	0	-	12	-	-	-
S234	AGATCCCGCC	8	-	8	-	-	-
S235	CAGTGCCGGT	8	-	9	-	-	-
S236	ACACCCCACA	8	-	7	-	6	-
S237	ACCGGCTTGT	10	-	0	-	9	-
S238	TGGTGGCGTT	8	-	7	-	8	-
S239	GGGTGTGCAG	9	-	7	-	9	-
S240	CAGCATGGTC	0	-	0	-	9	-
S241	ACGGACGTCA	0	-	0	-	7	-
S242	CTGAGGTCTC	0	-	0	-	7	-
S243	CTATGCCGAC	0	-	0	-	10	-
S245	TTGGCGGCCT	17	-	7	-	0	-
S246	ACCTTTGCGG	10	-	7	-	7	-
S247	CCTGCTCATC	0	-	0	-	6	-
S248	GGCGAAGGTT	0	-	0	-	3	-
S249	CCACATCGGT	6	-	6	-	0	-
S250	ACCTCGGCAC	10	-	12	-	-	-
S252	TCACCAGCCA	9	-	0	-	-	-
S254	TGGGTCCCTC	8	-	8	-	-	-
S255	ACGGGCCAGT	7	-	10	-	-	-
S256	CTGCGCTGGA	8	-	0	-	-	-
S257	ACCTGGGGAG	12	-	8	-	7	3
S258	GAGGTCCACA	0	-	10	-	-	-
S259	GTCAGTGCGG	13	-	12	-	5	-
S260	ACAGCCCCCA	17	-	12	-	5	-
S261	CTCAGTGTCC	10	-	12	-	3	-
S262	ACCCCGCCAA	6	-	0	-	0	-
S263	GTCCGGAGTG	4	-	0	-	5	-
S264	CAGAAGCGGA	5	-	5	-	7	-
S265	GGCGGATAAG	13	-	6	-	0	-
S266	AGGCCCGATG	9	-	8	-	6	-
S267	CTGGACGTCA	10	-	8	-	6	-
S268	GACTGCCTCT	13	-	12	-	3	-
S269	GTGACCGAGT	8	-	12	-	5	-
S270	TCGCATCCCT	13	-	14	-	5	-
S271	CTGATGCGTG	1	-	10	-	7	-
S272	TGGGCAGAAG	9	-	14	-	7	-
S273	CACAGCGACA	0	-	18	-	-	-
S275	ACACCGGAAC	0	-	10	-	-	-
S276	CAGCCTACCA	8	-	14	-	-	-
S277	GTCCTGGGTT	0	-	10	-	-	-
S278	TTCAGGGCAC	18	-	14	-	-	-
S279	CAAAGCGCTC	5	-	10	-	-	-
S280	TGTGGCAGCA	11	-	16	-	-	-

S281	GTGGCATCTC	11	-	16	-	-	-
S282	CATCGCCGCA	8	-	14	-	-	-
S283	ACAGCCTGCT	5	-	6	-	-	-
S284	GGCTGCAATG	8	-	0	-	-	-
S285	GGCTGCGACA	8	-	16	-	-	-
S286	AAGGCTCACC	13	-	14	-	-	-
S287	AGAGCCGTCA	15	-	16	-	-	-
S288	AGGCAGAGCA	8	-	5	-	-	-
S289	AGCAGCGCAC	13	-	0	-	-	-
S290	CAAACGTGGG	15	-	20	-	-	-
S291	AGACGATGGG	10	-	0	-	-	-
S293	GGGTCTCGGT	10	-	8	-	-	-
S294	GGTCGATCTG	8	-	8	-	-	-
S295	AGTCGCCCTT	8	-	21	-	-	-
S296	GGGCCAATGT	13	-	20	-	-	-
S297	GACGTGGTGA	13	-	20	-	-	-
S298	GTGGAGTCAG	6	-	12	-	-	-
S299	TGAGGGTCCC	0	-	13	-	-	-
S300	AGCCGTGGAA	10	-	0	-	-	-
Total number of bands		**741**	-	**783**	-	**206**	-

random primers were used for comparisons in RAPD patterns of J and A DNA amplifications. Thirty five primers could produce 206 clear bands. Primer S216 produced polymorphic PCR product with one fragment specific for Juvenile phase but was clearly absent in mature phase. Another primer S257 depicted polymorphic RAPD pattern between two phases. Amplified products of both phases showed discrimination by one prominent fragment of approximately 497bp length, which was present only in mature phase (Fig. 3).

Fig. 3. Comparison of amplified products by primer S216, from DNA of J and A phase of *Prunus persica*



Primer No. S216 Peach

The results of the present study demonstrate genomic differences in the nuclei of young leaves of J and A phase of crab apple and peach seedling trees. In crab-apple, three bands out of 741 and 783 in case of *Malus micromalus* and *Malus hupehensis,* respectively were polymorphic.The probability of bands showing variation was very low i.e. 0.404% and 0.383% in *Malus species* while in peach it was 1.45%. It is interesting to note that primer S216 produced RAPD-pattern from two phases of *Malus micromalus* and *Prunus persica.*Both species might have similar DNA sequences near by the motif that have gone under modifiction. In small genome of peach it was easier to detect the variation.

The finding of differential amplification in the RAPD assays between J and A phase DNAs, in crab apple and peach might suggest that a variation of genetic constitution has occurred between two phases, during transition. A few numbers of polymorphic bands between J and A DNAs might reveal that only a minor sequence change or structural variation of a near-by target site has taken place. It supports the view that a nuclear repatterning may occur in organs of an individual plant (Baluska, 1990). The results are in consistence with earlier observations (Zhang, 1997). In crab-apple and Olive shoot meristems, following the transition to adult phase, an increase in nuclear DNA content (Jin, 1993; Mazzuca *et al*., 1995) might be due to the fraction resulting from an over replication of sequences (Mazzuca *et al*., 1995; Bitonti *et al*., 1996). There is an increasing evidence of differential replication of DNA sequences during plant development leading to tissue specific genomic alteration has been reported in soybean leaf and root DNAs (Chen *et al*., 1994; 1997a).

During transition from J to A phase, shoot development is likely to be specified by a sequence of independently regulated, over-lapping programs that are able to modify the expression of the common set of processes (Poethig, 1990). The differential replication of DNA sequences may well be involved in the changes in morphogenic programs in the shoot apex (Bassi, 1990; Nagl, 1990; Mazzuca *et al*., 1995).

Selection of RAPDs as markers for such analysis has been performed because of their nature as random probes of the genome. RAPDs have been indicated to be reliable markers by a number of authors: as a mean for prediction of genetic diversity (Williams & St Clair, 1993), to determine variation of plants derived from somatic embryo genesis (Isabel, 1993) and to detect minor changes in genomic DNAs during development and ontogenesis within the single plant (Bitonti *et al*., 1996; Chen *et al*., 1997).

The other plausible reasons for DNA variation found in analysis would be owing to some flexible moieties in the genomes, serving as site for sequence rearrangement in response to diverse physiological condition in the organs of plant or such motifs were not replicated with high fidelity during cell division and differentiation and a very minor flux existed (Cullis, 1986; Shang & Wang, 1991; Bogani *et al*., 1996).

CONCLUSIONS

A series of observations reported in this paper seem to suggest that repatterning of genome, at least, in a few sequences, must have occurred in transition from J to A phase and might be related to program of morphological and physiological modifications. Phase specific DNA sequence variation might be further tagged for mapping, sequencing and tracing the possible genes related to differential replication of genomic DNA during the development of apple seedlings.

ACKNOWLEDGEMENT

This project was supported by Zhejiang Provencial Natural Science Foundation Grant No. 396072 and Cina Natural Science Foundation Grant No. 39770630.

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(Received 03 January 2000; Accepted 01 June 2000)