Marker-based analysis of genome structure and DNA methylation in a watermelon (*Citrullus lanatus*) ploidy series

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ABSTRACT. Polyploidization is a major driver of plant genome evolution and speciation. However, most investigations have focused on allopolyploidization, and little is known about the genetic and epigenetic impact imposed by autopolyploidization. In the present study, an autoploidy series (2x, 3x and 4x) in watermelon (*Citrullus lanatus*) was constructed. The genome structure changes and DNA methylation adjustments at CCGG sites in a genome-wide level were analyzed in the three different autoploidy watermelons by the ISSR, SRAP, and MSAP methods. Compared with diploids, no obvious genetic changes were discovered in autotriploids. Extensive changes occurred in autotetraploids in which sequence eliminations were predominant. However, analysis of DNA methylation level indicated that the total DNA methylation levels were not significantly different between diploid and autotetraploid. Autotriploid showed a trend of low DNA methylation level. It implied that the alterations of DNA methylation levels were not linear with the autopolyploidy level in watermelon. Further analysis suggested that about half of total sites experienced adjustments of DNA methylation patterns in different ploidy watermelons. Interestingly, autotriploid still showed an obvious trend of demethylation. These results indicated some differences from other investigated autopolyploid plants in autopolyploid watermelon. Autotriploid watermelon, in particular, showed unusual characters of genome structure and DNA methylation status, which may be closely associated with the odd-ploidy effect and the excellent traits present in triploid plants.

Keywords: Autopolyploidization; Allopolyploidization; DNA methylation; MSAP; Watermelon.

INTRODUCTION

Polyploidy is remarkably common in the plant kingdom, resulting either from duplication of the entire genome (autopolyploidy) or through combination of genomes from different parental species (allopolyploidy). The most popular estimate of the proportion of polyploid in flowering plants is about 70% (Masterson, 1994; Wendel, 2000). However, recent investigations indicate that almost all higher plants have undergone one or more polyploidization events during their evolutionary history, even many classic diploid plant species including model plants *Arabidopsis* and maize, are of polyploid origin (Helentjaris et al., 1988; The Arabidopsis Genome Initiative, 2000; Vision et al., 2000; Blanc et al., 2003; Ermolaeva et al., 2003). Thus, polyploidization has been regarded as one of the most important driving forces for plant genome evolution and speciation (Lewis, 1980; Soltis et al., 2004; Adams and Wendel, 2005). Moreover, because some polyploid plants possess excellent crop traits, such as increased abiotic and biotic stress tolerance, early flowering time, increased organ size and biomass, many important crops are also polyploids (Levin, 1983; Hilu, 1993; Liu and Wendel, 2002). The prominent evolutionary significance and practical value of polyploidy in agriculture underscore the importance of exploring the molecular nature of polyploidization (Chen and Ni, 2006).

A series of recent investigations focusing on allopolyploidy indicate that widespread genomic changes occur concomitantly with hybridization and genome doubling – the two most important events of allopolyploidization (Wendel, 2000; Ozkan et al., 2001; Liu and Wendel, 2002). It was first reported in *Brassica* that extensive genomic changes occurred in low-copy nuclear sequences in each of F_2 to F_5 generations of synthetic allotetraploids among different species (Song et al., 1995). Following that, rapid sequences elimination,

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gene silencing, transposon activation, and other genome or chromosome changes were widely detected (Feldman et al., 1997; Ozkan et al., 2001; Shaked et al., 2001; Kashkush et al., 2002; Madlung et al., 2002; Mishima et al., 2002; Pontes et al., 2004; Salina et al., 2004; Han et al., 2005; Petit et al., 2007), as well as the changes of epigenetic modification, especially DNA methylation (Matzke et al., 1999; Lee and Chen, 2001; Madlung et al., 2002), and gene expression in newly synthesized allopolyploid wheat (He et al., 2003), *Arabidopsis* (Wang et al., 2004; Wang et al., 2006) and *Brassica napus* (Osborn et al., 2003; Albertin et al., 2007). However, not all investigations have detected genomic changes in allopolyploid plants (Paun et al., 2007). A representative exception is allopolyploid cotton. A survey of approximately 22, 000 genomic loci from nine sets of synthesized allotetraploid and allohexaploid cotton by AFLP and MSAP fingerprinting suggested genomic stasis and lack of rapid DNA methylation changes at symmetric CCGG sites (Liu et al., 2001). The exceptions can also be found in *Brassica* (Axelsson et al., 2000) and sugarcane (Jannoo et al., 2007). These contrasting examples indicate that allopolyploidization in plants may involve a diverse array of molecular evolutionary strategies reflecting both genomic constituents and taxonomic considerations (Liu et al., 2001), further highlighting the complex molecular mechanism of allopolyploidization. However, despite considerable research attention, the underlying mechanisms remain poorly understood.

Compared to allopolyploidization, relatively few studies have been devoted to elucidating the consequences of autopolyploidization, in which only genome doubling occurs, partly because for many years autopolyploidy was considered extremely rare and maladaptive (Stebbins, 1950). Contradicting the view of autopolyploidy as an evolutionary dead-end, as asserted by Soltis et al. (2007), is the fact that the number of angiosperm species undergoing autopolyploidy has been grossly underestimated. In addition to *Zea diploperennis* and *Z. perennis*, at least five autopolyploids, i.e., *Tolmiea menziesii*, *Galax urceolata*, *Chamerion angustifolium*, *Heuchera grossulariifolia* and *Vaccinium corymbosum*, should be regarded as distinct species. Furthermore, several autopolyploid plants are successful crop species of major importance, including alfalfa, potato, cassava, watermelon, banana and apple cultivars, and sugar beet (Soltis and Soltis, 1993; Ming et al., 2001; NeSmith and Duval, 2001; Paterson, 2005). Substantial evidence indicates the potential of autopolyploids to create more new crop species or cultivars than their diploid progenitors and supports the need for further research to extend the utilization of autopolyploidy in agriculture.

In the present study, we constructed a set of autopolyploid (diploid, autotriploid and autotetraploid) watermelons (*Citrullus lanatus*) to detect possible genomic and epigenetic changes induced by autopolyploidization. Thereafter, we conducted a quantitative estimate of the frequency of genome-wide and epigenetic alterations using inter-sample sequence repeat (ISSR), sequence-related amplified polymorphism (SRAP) and methylation-sensitive amplified polymorphism (MSAP) analyses. Finally, we established the characteristic alterations in genome structure and DNA methylation at CCGG sites. Possible causes for the observed changes as well as differences from other autopolyploid or allopolyploid plants were discussed.

MATERIALS AND METHODS

Plant materials

The plant materials used in this study include homozygous diploid watermelon (*Citrullus lanatus*), which is an inbred line, newly synthesized autotriploid and autotetraploid watermelon, named JKR-1, JKR-2 and JKR-3, respectively. JKR-3 was created by colchicines-doubling the diploid as described by Jaskani et al. (2004) while the autotriploid JKR-2 was generated by crossing diploid, JKR-1 with the autotetraploid, JKR-3 (as the female recipient of pollen). The ploidy level of JKR-2 and JKR-3 was identified by chromosome number in root tip mitosis. All these materials were kindly provided by Prof. Dingliang-Jiao, Tianjin Vegetable Research Institute, Tianjin, China, and synchronously grown in a greenhouse under normal light conditions (day/light temperature of 25/19°C, 16 h photoperiod). After 10 days the young leaves from each of the three different ploidy watermelons were used for the extraction of genomic DNA.

Karyotype analysis

Chromosome preparations were prepared using the squash technique as described previously (Urdampilleta et al., 2006). In brief, tender root tips from germinated seeds of diploid watermelon (JKR-1) and predicted autotriploid and autotetraploid watermelons, were cut and pretreated with 2 mM 8-hydroxyquinoline for 4 h at 20°C, fixed in ethanol: acetic acid (3:1, V:V) for 10 h. Following that, all root tips were hydrolyzed in 1 N HCl for 10 min at room temperature and thoroughly rinsed with distilled water. After that the root tips were placed on a frozen slide, squashed, and then stained with Carbor fuchsin. Photographs were taken with a photomicroscope (Nikon80i, Japan). At least eight cells with good metaphase structures from a single plant were examined by this method. Only the individuals with the correct number of chromosomes (e.g., diploid with 2n=2x=22, triploid with 2n=3x=33 and tetraploid with 2n=4x=44) were considered for further investigation.

DNA extraction

Genomic DNA was extracted from young fresh leaves of eight individuals from JKR-1, JKR-2, and JKR-3, respectively, by the CTAB procedure (Murray and Thompson, 1980) with some modifications. In brief, young leaves (200 mg) were collected and immediately ground in liquid nitrogen, before the frozen power was added to 2

mL lysis buffer including 100 mM Tris-HCl (pH 8.0), 100 mM EDTA, 1.4 M NaCl, 0.2% β-mercaptoethanol, 2% PVP and 1×CTAB. After incubation at 56°C for 30 min, 2 mL phenol: chloroform: isoamyl alcohol (25:24:1, V:V:V) was added. The following steps were as described by our previous study (Wang et al., 2007; Gu et al., 2008).

ISSR and SRAP analysis

Thirty ISSR primers (Wang et al., 2008) were designed for PCR amplifications in JKR-1, JKR-2 and JKR-3. The optimized polymerase reaction conditions for 25 μl volume contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl$_2$, 200 μM dNTPs, 100 ng template DNA, 0.5 μM primer and 1 unit *Taq* polymerase (TaKaRa, Japan). Amplification was performed in an Eppendorf Master cycler gradient (Eppendorf Netheler-Hinz, Hamburg, Germany) following a touchdown PCR program: initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 45 s, annealing at a specific annealing temperature for 1 min and extension at 72°C for 1 min 30 s, with a final extension of 8 min at 72°C. The annealing was performed with touchdown decrements of 1°C starting at 65°C for the first 10 cycles followed by 25 cycles at 55°C. The amplified products were roughly analyzed by electrophoresis in a 1.5% agarose gel before being separated on a neutral 4% polyacrylamide gel (29:1, acryamide:bisacrylamide) to improve the resolution. The polyacrylamide electrophoresis was carried out in 1×TBE buffer at room temperature and run at 240 V for 3 h, and then the gel was stained with Silver Stain Kit (ATTO, Japan). Only clear and reproducible bands that appeared in two independent PCR amplifications with different batches of DNA isolations were considered for the following analysis.

For SRAP analysis, eight forward primers and ten reverse primers were used (Table 1). Each combination of forward and reverse primers was screened for its polymorphism and reproducibility. The primer pairs were excluded if their banding patterns were difficult to score or if they failed to amplify consistently. The PCR reaction mixtures (25 μl total volume) consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl$_2$, 200 μM dNTPs, 100 ng template DNA, 0.8 μl each of 10 mM forward and reverse primers and 1 unit *Taq* polymerase (TaKaRa, Japan). Amplifications were carried out following the program as described by Li and Qurios (2001). The amplified products were also roughly analyzed by 1.5% agarose gel, and then separated on a neutral 4% polyacrylamide gel as mentioned in ISSR analysis.

MSAP analysis

The protocol used for MSAP analysis was essentially as previously reported (Xiong et al., 1999; Xu et al., 2000; Sha et al., 2005; Dong et al., 2006), with modifications. Briefly, for each sample of watermelon materials, i.e., JKR-1, JKR-2 and JKR-3, aliquots (400 ng) of DNA were digested for 72 h at 37°C with 3 U *EcoR*I (TaKaRa, Japan) and 3 U *Hpa*II or *Msp*I (Promega, USA) in a final volume of 15 μl containing 1 × R-L buffer (0.05 mol/L Tris-HCl, 0.05 mol/L MgAc$_2$, 0.25 mol/L KAc). *Hpa*II and *Msp*I are a pair of isoschizomers that recognize the same tetranucleotide sequence 5′-CCGG (a prominent site for methylation in both plants and animals) but have different sensitivity to the methylation states of cytosines: *Hpa*II will not cut if either of the cytosines is fully (double-strand) methylated, whereas, *Msp*I will not cut if the external cytosine is fully-or hemi-(single-strand) methylated (Xu et al., 2000). The digested fragments were then ligated to the adapter by adding a 5 μl ligation mixture, containing 30 pmol *Hpa*II/*Msp*I adapters, 30 pmol *EcoR* I adapters, 0.4 mM ATP, 0.8 U T$_4$ DNA ligase (TaKaRa, Japan), and 1 μl 5 × R-L buffer in a final volume of 20 μl, and this ligation mixture was incubated at 16°C for 10-12 h. It was then diluted 1:10 with sterile water and used as the template for the pre-amplification with E$_{00}$ and H/M$_{00}$ primers. The reaction was performed for 25 cycles of 30 s denaturation at 94°C, 1 min annealing at 56°C, and 1 min extension at 72°C. The volume of pre-amplification was 20 μl, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl$_2$, 200 μM

Table 1. Forward and reverse primers using in SRAP assay.

Primer	Type	Sequence (5′-3′)	Primer	Type	Sequence (5′-3′)
Me5	Forward	TGAGTCCAAACCGGATA	Em1	Reverse	GACTGCGTACGAATTAAT
Me6	Forward	TGAGTCCAAACCGGAGC	Em2	Reverse	GACTGCGTACGAATTTGC
Me9	Forward	TGAGTCCAAACCGGAAT	Em3	Reverse	GACTGCGTACGAATTGAC
Me10	Forward	TGAGTCCAAACCGGACC	Em4	Reverse	GACTGCGTACGAATTAAC
Me11	Forward	TAGGTCCAAACCGGCTC	Em5	Reverse	GACTGCGTACGAATTGCA
Me12	Forward	TAGGTCCAAACCGGCGT	Em6	Reverse	GACTGCGTACGAATTCTT
Me14	Forward	TGAGTCCAAACCGGAGT	Em7	Reverse	GACTGCGTACGAATTGAT
Me15	Forward	TAGGTCCAAACCGGGTC	Em8	Reverse	GACTGCGTACGAATTTAA
			Em9	Reverse	GACTGCGTACGAATTTAT
			Em10	Reverse	GACTGCGTACGAATTTGC

"Me" indicated forward primers; "Em" indicated reverse primers.

dNTPs, 1 µl diluted ligation product, 0.5 µM of each E_{00} and H/M_{00} primers and 1 unit *Taq* polymerase (TaKaRa, Japan). These products were also diluted 10-fold with sterile water, and used as the template for selective amplification. Selective amplifications were conducted in a final volume of 20 µl, containing 1 µl of the diluted pre-amplification product and 0.5 µM each of E and H/M primers; the remaining components were the same as those in the pre-amplification reaction. The reaction was carried out as described by Xiong et al. (1999). The denatured PCR products were separated on a 6% denaturing polyacrylamide gel at 35 W for 2 h. The gel was then stained with silver (Chalhoub et al., 1997). Only clear and reproducible bands that appeared in two independent PCR amplifications [starting from the DNA digestion step, (i.e., the first step of MSAP) were scored].
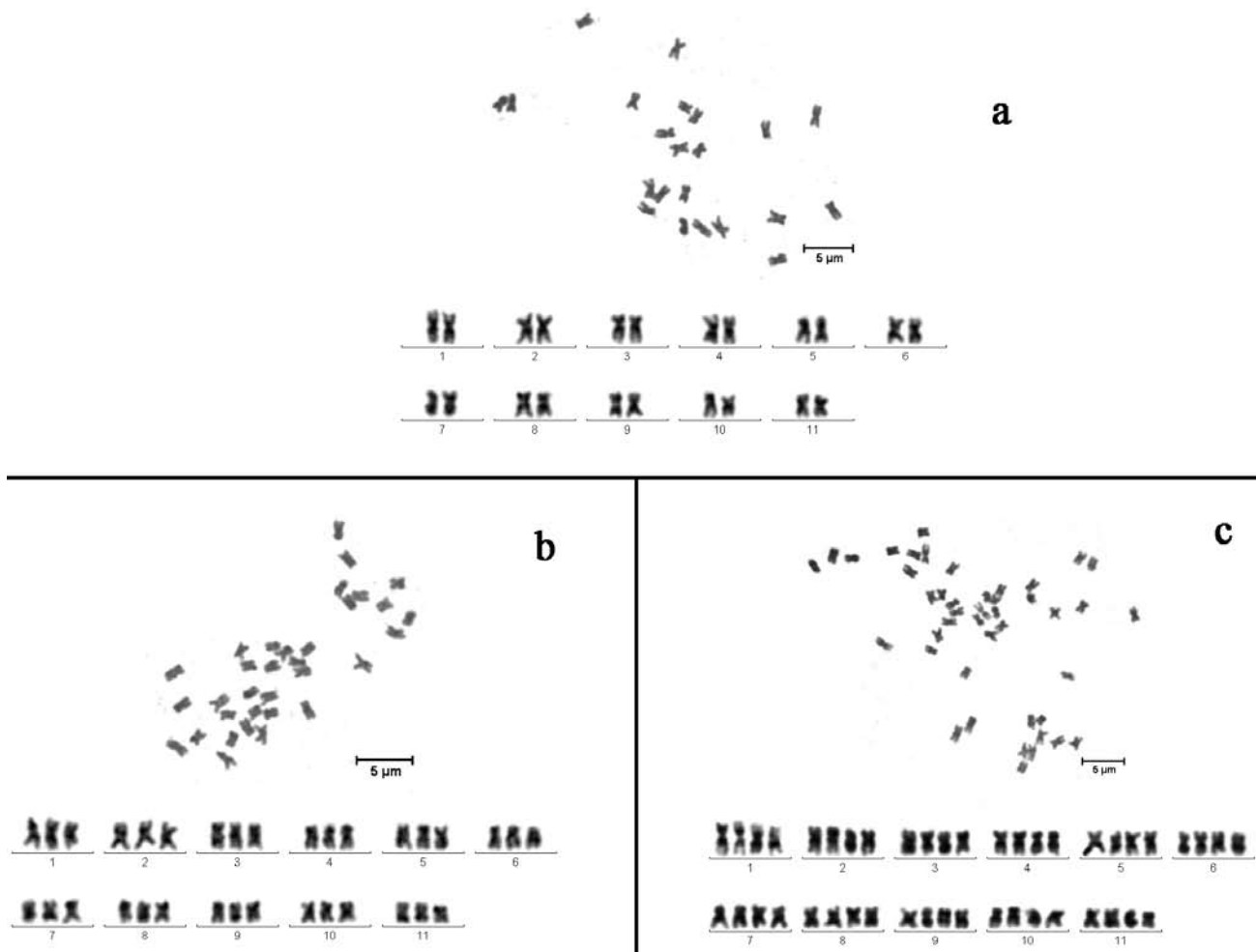
RESULTS

Validation of the ploidy level by Karyotype assay

The ploidy levels of all materials used in present study were determined by counting chromosome numbers in root cells. A total of ten individuals randomly selected from each of three different ploidy watermelons were tested. At least eight cells from two independent root tips of each sample were used to count the number of chromosomes. The results indicated that in each cell, the corresponding chromosome numbers were completely consistent with previous reports (Beevy and Kuriachan, 1996), implying that all materials were stable with respect to ploidy level, and no significant changes in chromosome structure had occurred (Figure 1).

Alteration in genomic structure detected by ISSR and SRAP assays

The ISSR assay generated approximately 1000 scored bands, for an average of 333 bands per ploidy sample, representing 328 prospective genetic loci. Partial results are presented in Figure 2. Among these 328 loci, 182 sites were simultaneously detected in three different ploidy samples, meaning that the remaining 146 were polymorphic, and the polymorphism frequency was thus 44.5% (146/328). A comparison of sites detected in 3x with those in 2x, showed that 17.4% (57/328) of sites were changed, of which 7.6% (25/328) sites were specifically

Figure 1. Karyotype of different ploidy watermelon. (a) diploid watermelon, JKR-1 (2n=2x=22); (b) autotriploid watermelon, JKR-2 (2n=3x=33); (c) autotetraploid watermelon, JKR-3 (2n=4x=44); Scale bar=5 µm.
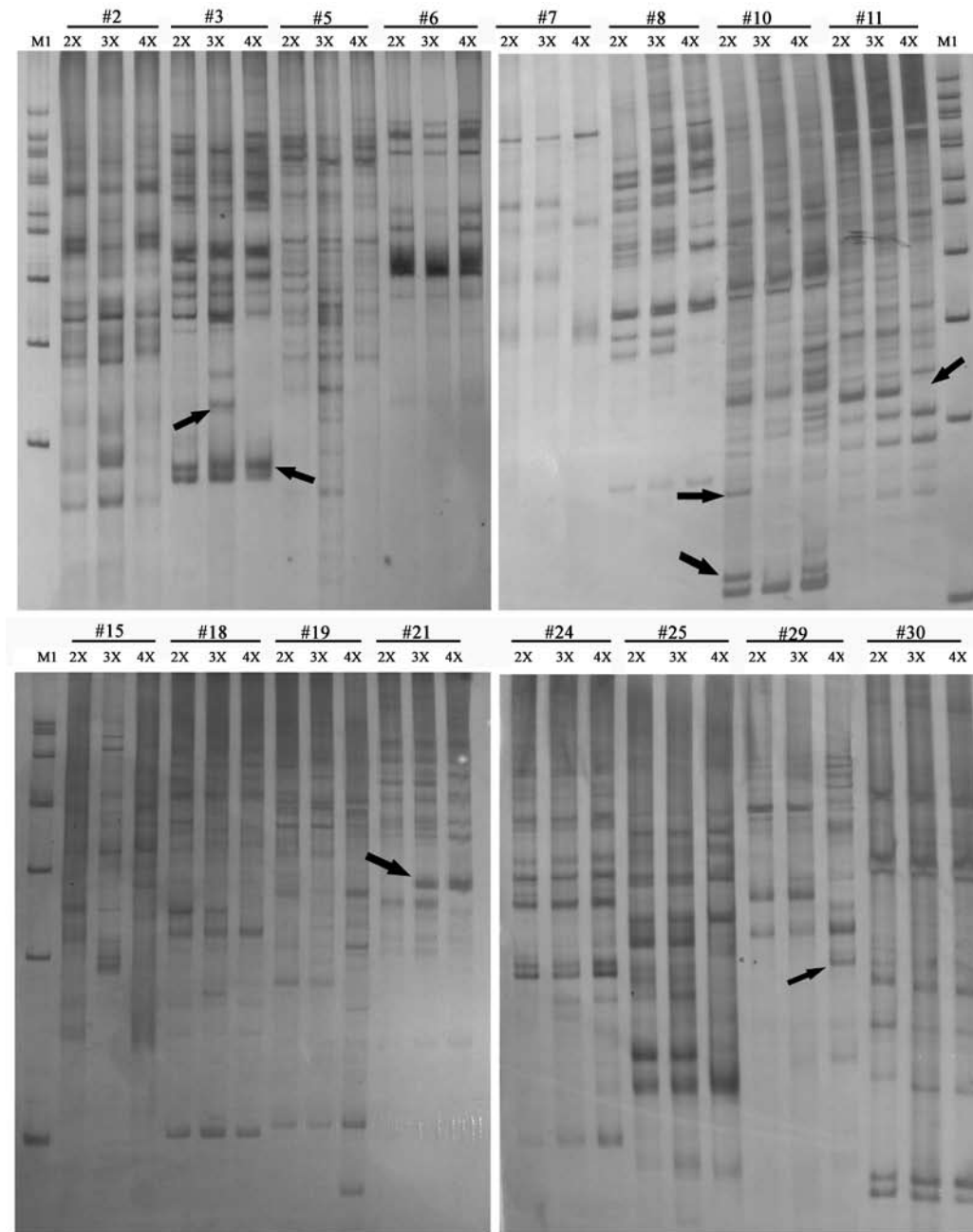
Figure 2. Amplification results of part ISSR primers in different ploidy watermelon (2x, 3x and 4x). "#" indicated the number of ISSR primers; "M1" indicated the molecular weight marker; Arrows indicated the different amplification patterns detected by ISSR primers in three different ploidy watermelon.

present in 3x; 9.8% (32/328) of sites were not detected. Comparisons between 3x and 4x, and between 4x and 2x were also conducted. In the 3x versus 4x comparison, 119 of 328 sites were found to be polymorphic, for a polymorphism ratio of 36.3%. Of these sites, 27.5% sites (90/328) were confirmed in 3x but not in 4x, and only 8.8% sites (29/328) were absent. In other words, a comparison of 4x with 3x showed that 27.5% sites were eliminated in 4x, indicating that the absent sites were predominant. The results obtained from a comparison of 4x with 2x indicated that 30 of 328 site (9.2%) were only detected

in 4x. However, the number of absent sites was 83. Thus the percentage of absent ratio sites (25.3%) was higher than that of present sites (9.2%). These data and additional details are presented in Table 2.

The SRAP assay generated a total of 1, 256 bands by 35 primer pairs, ranging in size from 100 bp to 2,000 bp representing 491 scored genetic loci. Partial results were presented in Figure 3. Among theses sites, 237 (48.3%) were polymorphic, and 254 were monomorphic. Pair-wise comparisons were carried out as for the ISSR analysis. A comparison between 3x and 2x indicated that only 35 sites

Table 2. Estimate of the genomic changes occurring in three difference ploidy (diploid, autotriploid and autotetraploid) watermelons by ISSR assay.

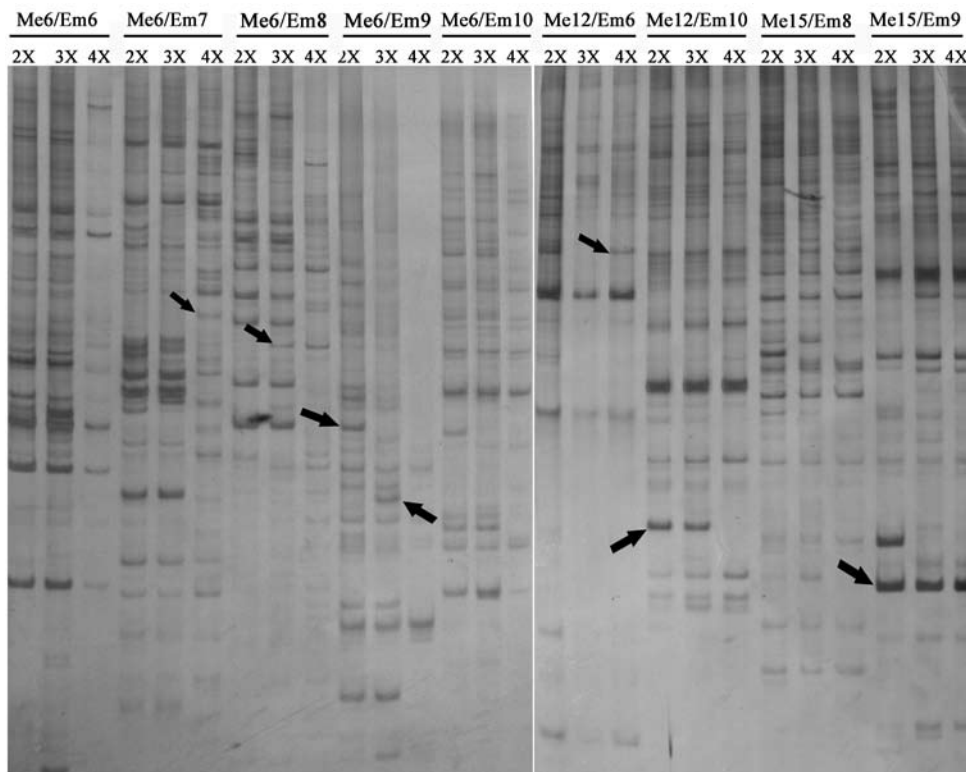|	Total sites	Identified sites	Polymorphic sites and corresponding ratio (%)	
2x-3x-4x	328	182	146 (44.5%)	
3x-2x	328	271	25$^+$ (7.6%)	57 (17.4%)
			32$^-$ (9.8%)	
3x-4x	328	209	90$^+$ (27.5%)	119 (36.3%)
			29$^-$ (8.8%)	
4x-2x	328	215	30$^+$ (9.2%)	113 (34.5%)
			83$^-$ (25.3%)	

"+" indicated the sites were present; "-" indicated the sites were absent.

(7.1%) were present, and 50 sites (10.2%) were specifically absent in 3x. A comparison between 3x with 4x indicated that 193 of 491 sites (39.3%) were polymorphic, of which 29.1% sites were present and only 10.2% sites were absent in 3x. The assay yielded similar results for a comparison between 4x and 2x. Thus, compared to 2x, only 9.2% sites (45/491) were present in 4x, a much small percentage than the 30.5% (150/491) of absent sites (Table 3).

Evaluation of DNA methylation levels and patterns at CCGG sites by MSAP assay

In MSAP assays, seven *Eco*RI and eight *Hpa*II/*Msp*I primer pairs were employed. Among 56 primer combinations, 23 pairs were selected for further study based on their ability to produce clear and completely reproducible bands in independent amplification reactions with different batches of DNA isolations. Approximately 1,200 bands were amplified in each of the three different ploidy watermelons, representing 647, 655 and 581 distinguishable genetic loci in 2x, 3x and 4x, respectively. On average, 60 bands (equal to 30 sites,) per primer pair were observed (Figure 4). Among all of these sites detected by MSAP method, the total methylation polymorphism ratios in 2x, 3x and 4x were 28.0%, 22.9% and 27.4%, respectively (Table 4). In 2x, 121 of 181 methylation sites were fully methylated, corresponding to a fully methylated ratio of 18.7% (121/647), while the hemi-methylated ratio was only 9.3% (60/647). In 3x, the fully methylated and

Figure 3. Amplification results of part SRAP primers in different ploidy watermelon (2x, 3x and 4x). Arrows indicated the different amplification patterns detected by SRAP primers in three different ploidy watermelon.

Table 3. Estimate of the genomic changes occurring in three difference ploidy (diploid, autotriploid and autotetraploid) watermelons by SRAP assay.

	Total sites	Identified sites	Polymorphic sites and corresponding ratio (%)	
2x-3x-4x	491	254	237 (48.3%)	
3x-2x	491	406	35^+ (7.1%)	85 (17.3%)
			50^- (10.2%)	
3x-4x	491	298	143^+ (29.1%)	193 (39.3%)
			50^- (10.2%)	
4x-2x	491	296	45^+ (9.2%)	195 (39.7%)
			150^- (30.5%)	

"+" indicated the sites were present; "-" indicated the sites were absent.

Table 4. DNA methylation levels in different ploidy (diploid, autotriploid and autotetraploid) watermelons, based on MSAP analysis using 23 primer pairs.

Watermelon	Total sites	Methylated CCGG sites		
		Fully methylated sites	Hemi-methylated sites	Total
Diploid	647	121 (18.7%)	60 (9.3%)	181 (28.0%)
Autotriploid	655	80 (12.2%)	70 (10.7%)	150 (22.9%)
Autotetraploid	581	82 (14.1%)	77 (13.3%)	159 (27.4%)
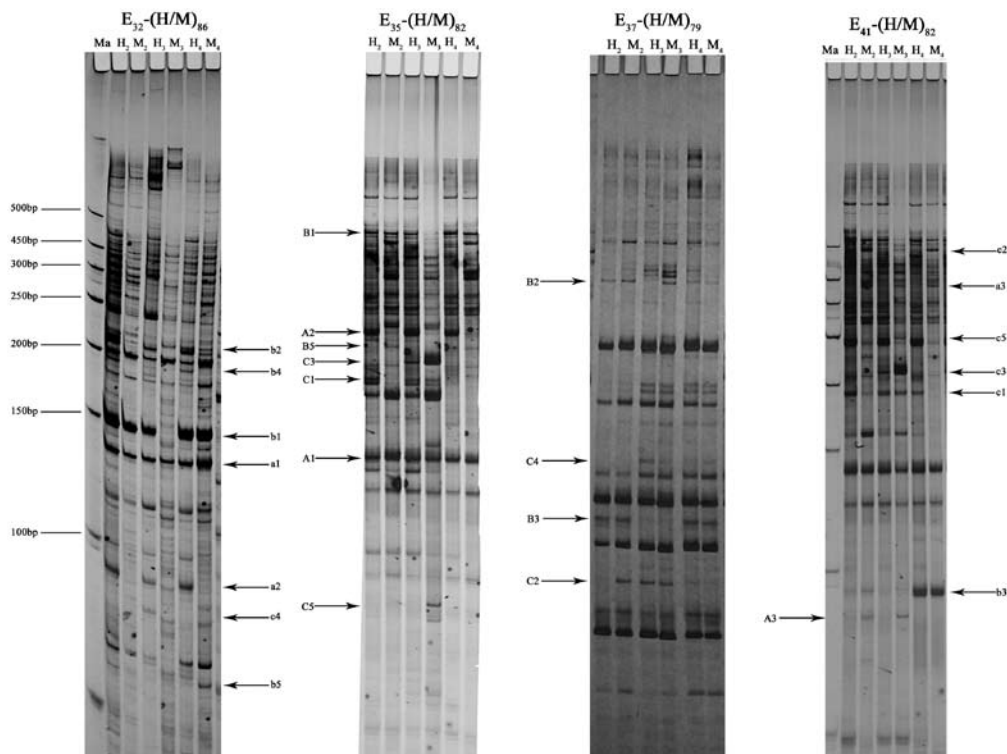
Figure 4. Different DNA methylation patterns detected in MSAP assay. "H_2, H_3 and H_4", respectively, indicated that total DNA of diploid (2x), autotriploid (3x) and autotetraploid (4x) was degested by *EcoR*I- *Hpa*II; "M_2, M_3 and M_4", respectively, indicated that total DNA of diploid (2x), autotriploid (3x) and autotetraploid (4x) was degested by *EcoR*I- *Msp*I; "Ma" indicated the molecular weight marker (50-bp ladder); Arrows indicated the different DNA methylation patterns in Table 5 and Table 6.

hemi-methylated ratios were 12.2% (80/655) and 10.7% (70/655), respectively. Similar results were observed in 4x, where the frequency of fully methylated sites was 14.1%, and the hemi-methylated ratio was 13.3% (Table 3). However, it should be noticed that several other states of DNA methylation—including unmethylated CCGG, full methylation of the external cytosine, and hemi-methylation of the internal cytosine—could not be unequivocally distinguished by the MSAP method. In some cases then, the methylation percentages will likely be underestimated (Cervera et al., 2002; Dong et al., 2006). This fact notwithstanding, MSAP's evaluation of two major methylation states at CCGG sites, i.e., full-methylation of the internal cytosine and hemi-methylation of internal cytosine, is still reliable and efficient (Knox and Ellis, 2001; Baurens et al., 2003; Guo et al., 2007).

All bands detected in three samples were scored as a binary character for absence (0) or presence (1). If a comparison between 3x or 4x with 2x was conducted, a total of four types of band patterns, including fifteen subtypes, was observed (Table 5). Type A showed methylation patterns that were unchanged in 3x or 4x

versus 2x. About half of the total patterns detected belonged to this type (3x vs. 2x: 56.5%; 4x vs 2x: 43.9%). Type B showed changes in patterns associated with the hypermethylation of certain sites in 3x or 4x versus 2x. A comparison of 3x with 2x, showed that 19.5% sites in 3x hypermethylated. However, a comparison between 4x and 2x indicated that the frequency of hypermethylation had increased to 33.9% in 4x. Type C represented samples in which demethylation events occurred in 3x or 4x versus 2x. A comparison of 3x with 2x, showed that 22.1% sites exhibited a decrease in methylation levels. A similar low methylation level (20.5%) was obtained in a comparison of 4x with 2x. Another type detected in the present study was type D, which corresponded to patterns unresolved by the MSAP method. Type D samples were not considered in subsequent assays. A similar analysis was conducted for 2x or 4x with 3x, and the detailed results are presented in Table 6. Based on the polymorphic band patterns, 35 fragments which may represent DNA methylation sites were successfully cloned and sequenced. Of these, six sequences indicated homology to known transposable elements by Blast analysis (data not shown).

Table 5. Patterns of DNA methylation and comparison between autotriploid or autotetraploid watermelon with diploid watermelon.

Pattern	3x or 4x		2x		No. and frequency of patterns		
	H	M	H	M	3x-2x	4x-2x	
A					415 (56.5%)	329 (43.9%)	
A1	1	1	1	1	382	296	
A2	1	0	1	0	10		=
A3	0	1	0	1	23	33	
B					143 (19.5%)	254 (33.9%)	
B1	1	0	1	1	39	59	
B2	0	1	1	1	25	42	
B3	0	0	1	1	20	69	↑
B4	0	0	1	0	36	45	
B5	0	0	0	1	23	39	
C					163 (22.1%)	153 (20.5%)	
C1	1	1	1	0	9	16	
C2	1	1	0	1	67	42	
C3	1	1	0	0	47	68	↓
C4	1	0	0	0	14	20	
C5	0	1	0	0	26	7	
D					14 (1.9%)	13 (1.7%)	
D1	0	1	1	0	6	5	
D2	1	0	0	1	8	8	≢
Total					735	749	

" = " indicated the patterns of DNA methylation at CCGG sites were unchanged; " ↑ " indicated the patterns of DNA methylation at CCGG sites were changed due to hypermethlyation events in 3x or 4x versus 2x; " ↓ " indicated the patterns of DNA methylation at CCGG sites were changed due to demethylation events in 3x or 4x versus 2x; " ≢ " indicated the alternations of DNA methylation patterns were unidentified by MSAP method.

Table 6. Patterns of DNA methylation and comparison between diploid or autotetraploid watermelon with autotriploid watermelon.

Pattern	2x or 4x		3x		No. and frequency of patterns		
	H	M	H	M	2x-3x	4x-3x	
a					415 (56.5%)	390 (53.8%)	
a1	1	1	1	1	382	348	
a2	1	0	1	0	10	18	=
a3	0	1	0	1	23	24	
b					143 (19.5%)	101 (13.9%)	
b1	1	1	1	0	39		
b2	1	1	0	1	25	31	
b3	1	1	0	0	20	43	↑
b4	1	0	0	0	36	20	
b5	0	1	0	0	23	7	
c					163 (22.1%)	228 (31.5%)	
c1	1	0	1	1	9	43	
c2	0	1	1	1	67	51	
c3	0	0	1	1	47	63	↓
c4	0	0	1	0	14	52	
c5	0	1	0	1	26	19	
d					14 (1.9%)	6 (0.8%)	
d1	1	0	0	1	6	6	
d2	0	1	1	0	8		≭
Total					735	725	

" **=** " indicated the patterns of DNA methylation at CCGG sites were unchanged; " ↑ " indicated the patterns of DNA methylation at CCGG sites were changed due to hypermethlyation events in 2x or 4x versus 3x; " ↓ " indicated the patterns of DNA methylation at CCGG sites were changed due to demethylation events in 2x or 4x versus 3x; " ≭ " indicated the alternations of DNA methylation patterns were unidentified by MSAP method.

DISCUSSION

As a major driver of plant genome evolution, polyploidization, has been studied by numerous research programs, with allopolyploidization, in particular, the focus of a number of studies. Considerable evidence indicates that allopolyploidization events can result in rapid genetic changes (Song et al., 1995; Han et al., 2003; Madlung et al., 2005; Petit et al., 2007). One conceivable explanation for these changes is the "genomic shock" hypothesis proposed by McClintock (1984). In this hypothesis, when allopolyploidization events occur, two or more divergent, but related, genomes are brought together in a single cell nucleus. The cytoplasmic-nuclear equilibrium established in the progenitor is destroyed and different genomic interactions take place. Reestablishing the cytoplasmic-nuclear balance and stabilizing multiple genomes in a single nucleus may necessitate certain adjustments for allopolyploid plants. However, previous investigations indicated that genetic alterations were not prevalent in autopolyploid plants. In addition, the function and the expression patterns of duplicated genes were also less affected (Albertin et al., 2005; Wang et al., 2006; Stupar et al., 2007). Interestingly, the results of our present ISSR and SRAP analyses of a watermelon autoploidy series demonstrated some inconsistencies with respect to the literature. As in previous reports, the present data indicated that no obvious genomic structure changes had occurred in the autotriploids (Table 2, Table 3). However, comparisons of the autotetraploid with diploid or autotriploid yielded the opposite results, indicating that the genomic structure in the autotetraploid had dramatically changed (Table 2, Table 3). Approximate 40% of total detected sites showed these changes; although some sites were specifically present, more sites were specifically eliminated in the autotetraploid. As a result, the genomic changes that occurred in 4x watermelon resembled those in most allopolyploid plants, where site elimination events are prominent. These data essentially indicated that, although autotriploid and autotetraploid watermelons were both polyploids, the characteristic genomic changes were different. Taken together with the exceptions presented by allopolyploid cotton (Liu et al., 2001), sugarcane (Jannoo et al., 2007), and the review of Paun et al. (2007), the

results presented here allow us to boldly predict that the ability to respond to "polyploidization"—that is, "genomic shock"—differs from plant to plant and may be closely associated with the polyploidy level. A similar opinion is expressed by Soltis and Soltis (1999). Accordingly, different plants may possibly possess their own critical ploidy value. If this is the case, at a polyploidy level below the critical plant-specific ploidy value, polyploid plants need not adjust their genomic structure on a large scale. Once the polyploidy level crosses the critical ploidy value, rapid genetic changes are required to stabilize the genomes and reconcile nuclear-cytoplasmic compatibility. One of the major genetic alterations is rapid DNA sequence elimination, which allows polyploid plants to reduce some redundant sequences and possibly reestablish stable cytoplasmic-nuclear interactions. Based on this hypothesis, allopolyploidization might give rise to more extensive "genomic shock" for most plants than would autopolyploidization. Consequently, the critical ploidy value is low. Once the allopolyploidization events occur, the genetic changes are necessary. The apparent exceptions of allopolyploid cotton, sugarcane and others, may be explained by arguing that the tolerance of the divergent genomes to nuclear or cytoplasmic-nuclear interactions in these plant species is sufficient to allow them to cope with "genomic shock." Under these conditions, rapid genomic structural alterations or other extensive genetic changes are apparently unnecessary, implying that the critical ploidy value, if it exists, may be higher in these plants. However, the question of whether the concept of a critical ploidy value can truly be applied to the whole plant kingdom awaits further investigations into the character of the genomic alterations that accompany polyploidization. Based on our hypothesis, during autopolyploidization in watermelon, triploidy may present the critical ploidy value. Accordingly, the ploidy levels above this value, such as autotetraploidy, provoke rapid genomic changes while ploidy levels less than or equal to this value, including autotriploidy, are not accompanied by dramatic genomic changes.

Unlike the results of genomic structure analysis, the estimates of DNA methylation levels and patterns suggested that the frequency of DNA methylation sites was similar between diploid and autotetraploid, while autotriploid exhibited a clear trend toward lower methylation levels, implying that the level of DNA methylation at CCGG sites was non-linearly related to ploidy level in watermelon. Further analysis of DNA methylation patterns indicated that about half of the observed sites underwent alterations in DNA methylation patterns during autopolyploidization, suggesting that the changes related to DNA methylation modification were more extensive and visible than the alterations of genomic structure. Of particular interest, no extensive alterations in genomic level occurred in autotriploid versus diploid watermelon, but the changes in DNA methylation patterns were obvious. DNA methylation, commonly occurring at the C5 position of cytosine residues

predominantly in symmetric CG and CNG sequences (Finnegan et al., 1998) or in many cases observed in non-symmetric CNN sites (Steward et al., 2002) (where N is A, T or C), is one of the key epigenetic regulation mechanisms in plants and is closely associated with gene silencing, transposon activation, and genomic imprinting (Wassenegger, 2000). This may imply that priority of genetic changes, epigenetic regulation (i.e., alterations of DNA methylation levels and patterns) occurs in response to the "genomic shock" resulting from allopolyploidization or autopolyploidization. In other words, below a certain ploidy level, polyploid plants need not extensively change their genomic structure, and can rely instead on changes in epigenetic regulation to maintain the genomic stability. However, if the ploidy level exceeds the tolerance limits of the plant, both rapid genomic changes and epigenetic regulation may be necessary. It suggests that epigenetic modification is an early event in plant evolution and is significant for the stability of genomic structure and control of gene expression.

The autotriploid is still an interesting subject of study, despite the fact that no extensive genomic structural changes occurred relative to the diploid (Table 2, Table 3). For example, the autotriploid showed lower DNA methylation levels compared to the diploid and the autotetraploid (Table 4). Moreover, demethylation events in the autotriploid were predominant (Table 5, Table 6). As we know, low DNA methylation levels and demethylation in plants are generally associated with the expression of genes; in contrast, the repression of gene expressions is always associated with high DNA methylation status (Diéguez et al., 1997; Cao and Jacobsen, 2002; Wada et al., 2004). Given this, the low DNA methylation levels and the trend of demethylation of numerous sites in autotriploid watermelon may suggest up-regulation of many genes. This phenomenon may be explained by the "odd-ploidy effect" first observed by Guo et al. (1996) (Auger et al., 2005; Stupar et al., 2007). It may also be associated with the formation of several excellent traits, such as rapid growth, a large vegetative organ and seedlessness in the autotriploid watermelon, as well as in other triploid plants. However, numerous investigations have been focused on allopolyploidization and even-ploidy plants. Little is known about the odd-ploidy plants, specifically, triploids, which often hold excellent traits in some crops (Hamada, 1963; Kagan-Zur et al., 1990; Bhojwani and Razdan, 1996; Sreekumari et al., 1999; Thomas et al., 2000). Therefore, more investigations focused on odd-ploidy plants are required to further elucidate the molecular mechanism of odd-ploidy effects, especially the triploidy effect.

LITERATURE CITED

Adams, K.L. and J.F. Wendel. 2005. Polyploidy and genome evolution in plants. Curr. Opin. Plant Biol. **8:** 135-141.

Albertin, W., P. Brabant, O. Catrice, F. Eber, E. Jenczewski, A-M. Chèvre, and H. Thiellement. 2005. Autopolyploidy in cabbage (*Brassica oleracea* L.) does not alter significantly the proteomes of green tissues. Proteomics **5:** 2131-2139.

Albertin, W., K. Alix, T. Balliau, P. Brabant, M. Davanture, C. Malosse, B. Valot, and H. Thiellement. 2007. Differential regulation of gene products in newly synthesized *Brassica napus* allotetraploids is not related to protein function nor subcellular localization. BMC Genomics **8:** 56.

Auger, D.L., A.D. Gray, T.S. Ream, A. Kato, E.H. Jr Coe, and J.A. Birchler. 2005. Nonadditive gene expression in diploid and triploid hybrids of maize. Genetics **169:** 389-397.

Axelsson, T., C.M. Bowman, A.G. Sharpe, D.J. Lydiate, and U. Lagercrantz. 2000. Amphidiploid *Brassica juncea* contains conserved progenitor genomes. Genome **43:** 679-688.

Baurens F-C., F. Bonnot, D. Bienvenu, S. Causse, and T. Legavre. 2003. Using SD-AFLP and MSAP to assess CCGG methylation in the banana genome. Plant Mol. Biol. Rep. **21:** 339-348.

Beevy, S.S. and P. Kuriachan. 1996. Chromosome numbers of south Indian Cucurbitaceae and a note on the cytological evolution in the family. J. Cytol. Genet. **31:** 65-71.

Bhojwani, S.S. and M.K. Razdan. 1996. Plant tissue culture: theory and practice, a revised edition. Elsevier, Amsterdam, pp. 1-766.

Blanc, G., K. Hokamp, and K.H. Wolfe. 2003. A recent polyploidy superimposed on older large-scale duplications in the *Arabidopsis* genome. Genome Res. **13:** 137-144.

Cao, X. and S.E. Jacobsen. 2002. Role of the *Arabidopsis* DRM methyltransferases in de novo DNA methylation and gene silencing. Curr. Biol. **12:** 1138-1144.

Cervera, M.T., L. Ruiz-Garcia, and J.M. Martinez-Zapater. 2002. Analysis of DNA methylation in *Arabidopsis thaliana* based on methylation sensitive AFLP markers. Mol. Genet. Genomics **268:** 543-552.

Chalhoub, B.A., S. Thibault, V. Laucou, C. Rameau, H. Höfte, and R. Cousin. 1997. Silver staining and recovery of AFLP amplification products on large denaturing polyacrylamide gels. Biotechniques **22:** 216-220.

Chen, Z.J. and Z. Ni. 2006. Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. BioEssays **28:** 240-252.

Diéguez, M.J., M. Bellotto, K. Afsar, O. Mittelsten Scheid, and J. Paszkowski. 1997. Methylation of cytosines in nonconventional methylation acceptor sites can contribute to reduced gene expression. Mol. Gen. Genet. **253:** 581-588.

Dong, Z.Y., Y.M. Wang, Z.J. Zhang, Y. Shen, X.Y. Lin, X.F. Ou, F.P. Han, and B. Liu. 2006. Extent and pattern of DNA methylation alteration in rice lines derived from introgressive hybridization of rice and *Zizania latifolia* Griseb. Theor. Appl. Genet. **113:** 196-205.

Ermolaeva, M.D., M. Wu, J.A. Eisen, and S.L. Salzberg. 2003. The age of the *Arabidopsis thaliana* genome duplication. Plant Mol. Biol. **51:** 859-866.

Feldman, M., B. Liu, G. Segal, S. Abbo, A.A. Levy, and J.M. Vega. 1997. Rapid elimination of low-copy DNA sequences in polyploid wheat: A possible mechanism for differentiation of homoeologous chromosomes. Genetics **147:** 1381-1387.

Finnegan, E.J., R.K. Genger, W.J. Peacock, and E.S. Dennis. 1998. DNA methylation in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49:** 223-247.

Gu, Y., Q.C Zhao, D.L. Sun, and W.Q. Song. 2008. A genetic linkage map based on AFLP and NBS markers in cauliflower (*Brassica oleracea* var. *botrytis*). Bot. Stud. **49:** 93-99.

Guo, M., D. Davis, and J.A. Birchler. 1996. Dosage effects on gene expression in a maize ploidy series. Genetics **142:** 1349-1355.

Guo, W.L., R. Wu, Y.F. Zhang, X.M. Liu, H.Y. Wang, L. Gong, Z.H. Zhang, and B. Liu. 2007. Tissue culture-induced locus-specific alteration in DNA methylation and its correlation with genetic variation in *Codonopsis lanceolata* Benth. et Hook. f. Plant Cell Rep. **26:** 1297-1307.

Hamada, S. 1963. Polyploid mulberry tree in practice. Indian J. Ser. **1:** 3-4.

Han, F.P., G. Fedak, T. Ouellet, and B. Liu. 2003. Rapid genomic changes in interspecific and intergeneric hybrids and allopolyploids of *Triticeae*. Genome **46:** 716-723.

Han, F., G. Fedak, W. Guo, and B. Liu. 2005. Rapid and repeatable elimination of a parental genome-specific DNA repeat (pGc1R-1a) in newly synthesized wheat allopolyploids. Genetics **170:** 1239-1245.

He, P., B.R. Friebe, B.S. Gil, and J.M. Zhou. 2003. Allopolyploidy alters gene expression in the highly stable hexaploid wheat. Plant Mol. Biol. **52:** 401-414.

Helentjaris, T., D. Weber, and S. Wright. 1988. Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. Genetics **118:** 353-363.

Hilu, K.W. 1993. Polyploidy and the evolution of domesticated plants. Am. J. Bot. **80:** 1494-1499.

Jannoo, N., L. Grivet, N. Chantret, O. Garsmeur, J.C. Glaszmann, P. Arruda1, and A. D'Hont. 2007. Orthologous comparison in a gene-rich region among grasses reveals stability in the sugarcane polyploid genome. The Plant J. **50:** 574-585.

Jaskani, M.J., S.W. Kwon, G.C. Koh, Y.C. Huh, and B.R. Ko. 2004. Induction and characterization of tetraploid

watermelon. J. Kor. Soc. Hort. Sci. **45:** 60-65.

Kagan-Zur, V., D. Mills, and Y. Mizrahi. 1990. Callus formation from tomato endosperm. Acta Hortic. **280:** 139-142.

Kashkush, K., M. Feldman, and A.A. Levy. 2002. Gene loss, silencing and activation in a newly synthesized wheat allotetraploid. Genetics **160:** 1651-1659.

Knox, M.R. and T.H.N. Ellis. 2001. Stability and inheritance of methylation states at PstI sites in Pisum. Mol. Genet. Genomics **265:** 497-507.

Lee, H-S. and Z.J. Chen. 2001. Protein-coding genes are epigenetically regulated in *Arabidopsis* polyploids. PNAS **98(12):** 6753-6758.

Levin, D.A. 1983. Polyploidy and novelty in flowering plants. Am. Nat. **122:** 1-25.

Lewis, W.H. 1980. Polyploidy: Biological Relevance. New York: Plenum Press, 583 pp.

Li, G. and C.F. Qurios. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor. Appl. Genet. **103:** 455-461.

Liu, B., C.L. Brubaker, G. Mergeai, R.C. Cronn, and J.F. Wendel. 2001. Polyploid formation in cotton is not accompanied by rapid genomic changes. Genome **44:** 321-330.

Liu, B. and J.F. Wendel. 2002. Non-mendelian phenomena in allopolyploid genome evolution. Curr. Genomics **3:** 489-505.

Madlung, A., A.P. Tyagi, B. Watson, H. Jiang, T. Kagochi, R.W. Doerge, R. Martienssen, and L. Comai. 2005. Genomic changes in synthetic *Arabidopsis* polyploids. The Plant J. **41:** 221-230.

Madlung, A., R.W. Masuelli, B. Watson, S.H. Reynolds, J. Davison, and L. Comai. 2002. Remodeling of DNA methylation and phenotypic and transcriptional changes in synthetic *Arabidopsis* allotetraploids. Plant Physiol. **129:** 733-746.

Masterson, J. 1994. Stomatal size in fossil plants: Evidence for polyploidy in majority of angiosperms. Science **264:** 421-424.

Matzke, M.A., O.M. Scheid, and A.J.M. Matzke. 1999. Rapid structural and epigenetic changes in polyploid and aneuploid genomes. BioEssays **21:** 761-767.

McClintock, B. 1984. The significance of responses of the genome to challenge. Science **226:** 792-801.

Ming, R., S.C. Liu, P.H. Moore, J.E. Irvine, and A.H. Paterson. 2001. QTL analysis in a complex autopolyploid: Genetic control of sugar content in sugarcane. Genome Res. **11:** 2075-2084.

Mishima, M., N. Ohmido, K. Fukui, and T. Yahara. 2002. Trends in site-number change of rDNA loci during polyploid evolution in *Sanguisorba* (Rosaceae). Chromosoma **110:** 550-558.

Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high weight plant DNA. Nucleic Acids Res. **8:** 4231-4235.

NeSmith, D.S. and J.R. Duval. 2001. Fruit set of triploid

watermelons as a function of distance from a diploid pollinizer. HortSci. **36:** 60-61.

Osborn, T.C., J.C. Pires, J.A. Birchler, D.L. Auger, Z.J. Chen, H-S. Lee, L. Comai, A. Madlung, R.W. Doerge, V. Colot, and R.A. Martienssen. 2003. Understanding mechanisms of novel gene expression in polyploids. Trends Genet. **19(3):** 141-147.

Ozkan, H., A.A. Levy, and M. Feldman. 2001. Allopolyploidy induced rapid genomic evolution in the wheat (*Aegilops-Triticum*) group. Plant Cell **13:** 1735-1747.

Paterson, A.H. 2005. Polyploidy, evolutionary opportunity, and crop adaptation. Genetica **123:** 191-196.

Paun, O., M.F. Fay, D.E. Soltis, and M.W. Chase. 2007. Genetic and epigenetic alterations after hybridization and genome doubling. Taxon **56:** 649-656.

Petit, M., K. Yoong Lim, E. Julio, C. Poncet, F.D. Borne, A. Kovarik, A.R. Leitch, M-A. Grandbastien, and C. Mhiri. 2007. Differential impact of retrotransposon populations on the genome of allotetraploid tobacco (*Nicotiana tabacum*). Mol. Genet. Genomics **278:** 1-15.

Pontes, O., N. Neves, M. Silva, M.S. Lewis, A. Madlung, L. Comai, W. Viegas, and C.S. Pikaard. 2004. Chromosomal locus rearrangements are a rapid response to formation of the allotetraploid *Arabidopsis suecica* genome. PNAS **101:** 18240-18245.

Salina, E.A., O.M. Numerova, H. Ozkan, and M. Feldman. 2004. Alterations in subtelomeric tandem repeats during early stages of allopolyploidy in wheat. Genome **47:** 860-867.

Sha, A.H., X.H. Lin, J.B. Huang, and D.P. Zhang. 2005. Analysis of DNA methylation related to rice adult plant resistance to bacterial blight based on methylation-sensitive AFLP (MSAP) analysis. Mol. Gen. Genomics **273:** 484-490.

Shaked, H., K. Kashkush, H. Ozkan, M. Feldman, and A.A. Levy. 2001. Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell **13:** 1749-1759.

Soltis, D.E. and P.S. Soltis. 1993. Molecular data and dynamic nature of polyploidy. Crit. Rev. Pl. Sci. **12:** 243-273.

Soltis, D.E. and P.S. Soltis. 1999. Polyploidy: recurrent formation and genome evolution. Trends Ecol. Evol. **14:** 348-352.

Soltis, D.E., P.S. Soltis, D.W. Schemske, J.F. Hancock, J.N. Thompson, B.C. Husband, and W.S. Judd. 2007. Autopolyploidy in angiosperms: have we grossly underestimated the number of species? Taxon **56:** 13-30.

Soltis, D.E., P.S. Soltis, and J.A. Tate. 2004. Advances in the study of polyploidy since plant speciation. New Phytol. **161:** 173-191.

Song, K., P. Lu, K. Tang, and T.C. Osborn. 1995. Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc. Natl. Acad. Sci. USA **92:** 7719-7723.

Sreekumari, M.T., J.S. Jos, and S.G. Nair. 1999. 'Sree Harsha':

A superior triploid hybrid in cassava. Euphytica **106:** 1-6.

Stebbins, G.L. 1950. Variation and Evolution in Plants. Columbia University Press, New York.

Steward, N., M. Ito, Y. Yamaguchi, N. Koizumi, and H. Sano. 2002. Periodic DNA methylation in maize nucleosomes and demethylation by environmental stress. J. Biol. Chem. **277:** 37741-37746.

Stupar, R.M., P.B. Bhaskar, B.S. Yandell, W.A. Rensink, A.L. Hart, S. Ouyang, R.E. Veilleux, J.S. Busse, R.J. Erhardt, C.R. Buell, and J. Jiang. 2007. Phenotypic and transcriptomic changes associated with potato autopolyploidization. Genetics **176:** 2055-2067.

The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant, *Arabidopsis thaliana*. Nature **408:** 796-815.

Thomas, T.D., A.K. Bhatnagar, and S.S. Bhojwani. 2000. Production of triploid plants of mulberry (*Morus alba* L.) by endosperm culture. Plant Cell Rep. **19:** 395-399.

Urdampilleta, J.D., M.S. Ferrucci, J.M.D. Torezan, and A.L.L. Vanzela. 2006. Karyotype relationships among four South American species of Urvillea (Sapindaceae: Paullinieae). Pl. Syst. Evol. **258:** 85-95.

Vision, T.J., D.G. Brown, and S.D. Tanksley. 2000. The origins of genomic duplications in *Arabidopsis*. Science **290:** 2114-2117.

Wada, Y., K. Miyamoto, T. Kusano, and H. Sano. 2004. Association between up-regulation of stress-responsive genes and hypomethylation of genomic DNA in tobacco plants. Mol. Gen. Genomics **271:** 658-666.

Wang, C., X. Chen, H. Li, and W. Song. 2007. RNA editing analysis of mitochondrial *nad3/rps12* genes in cytoplasmic male sterility and fertile cauliflower (*Brassica oleracea* var. *botrytis*) by cDNA-SSCP. Bot. Stud. **48(1):** 13-23.

Wang, C.G., X.Q. Chen, H. Li, Q.C. Zhao, D.L. Sun, and W.Q. Song. 2008. Isolation and identification of specific sequences correlated to cytoplasmic male sterility and fertile maintenance in cauliflower (*Brassica oleracea* var. *botrytis*). Fen Zi Xi Bao Sheng Wu Xue Bao. **41:** 19-27.

Wang, J., L. Tian, A. Madlung, H-S. Lee, M. Chen, J.J. Lee, B. Watson, T. Kagochi, L. Comai, and Z.J. Chen. 2004. Stochastic and epigenetic changes of gene expression in *Arabidopsis* polyploids. Genetics **167:** 1961-1973.

Wang, J., L. Tian, H-S. Lee, N.E. Wei, H. Jiang, B. Watson, A. Madlung, T.C. Osborn, R.W. Doerge, L. Comai, and Z.J. Chen. 2006. Genomewide nonadditive gene regulation in *Arabidopsis* allotetraploids. Genetics **172:** 507-517.

Wassenegger, M. 2000. RNA-directed DNA methylation. Plant Mol. Biol. **43:** 203-220.

Wendel, J.F. 2000. Genome evolution in polyploids. Plant Mol. Biol. **42:** 225-249.

Xiong, L.Z., C.G. Xu, M.A. Saghai Maroof, and Q. Zhang. 1999. Patterns of cytosine methylation in an elite rice hybrid and its parental lines, detected by a methylation-sensitive amplifcation polymorphism technique. Mol. Gen. Genet. **261:** 439-446.

Xu, M., X. Li, and S.S. Korban. 2000. AFLP-based detection of DNA methylation. Plant Mol. Biol. Rep. **18:** 361-368.

不同倍性西瓜 (*Citrullus lanatus*) 基因組結構和 DNA 甲基化基於標記技術的分析

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　　多倍化是植物基因組進化和物種形成的主要驅動力,在植物界中廣泛存在。然而,多數研究都聚焦在植物的異源多倍化過程。有關植物同源多倍化過程中的遺傳及表觀遺傳變化報導很少。為此,本研究以西瓜的同源多倍體系列(2x、3x、4x)為材料,利用 ISSR、SRAP 和 MSAP 方法探究西瓜同源多倍化過程中的基因組結構變化及全基因組內 CCGG 位點處的 DNA 甲基化水準與模式變化特徵。結果表明,儘管與二倍體相比,在三倍體西瓜內未檢測到明顯的基因組結構變化,而四倍體西瓜的基因組結構變化十分顯著,並且變化以 DNA 序列消減為主。有趣地是,二倍體與四倍體西瓜相比 DNA 甲基化水準卻未發生大規模的調整,而三倍體西瓜顯示出較為明顯的低 DNA 甲基化水準趨勢,表明西瓜同源多倍化過程中其基因組 DNA 甲基化水準變化與其倍性不成線性關係。進一步分析證明,在檢測到的遺傳位點中,約有一半的位點 DNA 甲基化模式在不同倍性的西瓜中發生了調整。三倍體西瓜依然顯示出明顯地去甲基化趨勢。綜上結果證明,西瓜同源多倍化過程中發生的遺傳和表觀遺傳改變與已報導的一些植物的同源多倍化有所不同,特別是三倍體西瓜,其表現出獨特的基因組結構及 DNA 甲基化狀態變化特徵,而這可能與植物的奇倍性效應及三倍體西瓜優良性狀的形成密切相關。

關鍵詞:同源多倍化;異源多倍化;DNA 甲基化;MSAP; 西瓜。