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Retrotransposon- and microsatellite sequence-associated genomic changes in early generations of a newly synthesized allotetraploid *Cucumis* × *hytivus* Chen & Kirkbride

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Abstract Allopolyploidization is considered an essential evolutionary process in plants that could trigger genomic shock in allopolyploid genome through activation of transcription of retrotransposons, which may be important in plant evolution. Two retrotransposon-based markers, inter-retrotransposon amplified polymorphism and retrotransposon-microsatellite amplified polymorphism and a microsatellite-based marker, inter simple sequence repeat were employed to investigate genomic changes in early generations of a newly synthesized allotetraploid *Cucumis* × *hytivus* Chen & Kirkbride (2n = 4*x* = 38) which was derived from crossing between cultivated cucumber *C. sativus* L. (2n = 2*x* = 14) and its wild relative *C. hystrix* Chakr. (2n = 2*x* = 24). Extensive genomic changes were observed, most of which involved the loss of parental DNA fragments and gain of novel fragments in the allotetraploid. Among the 28 fragments examined, 24 were lost while four were novel, suggesting that DNA sequence elimination is a relatively frequent event during polyploidization in *Cucumis*. Interestingly, of the 24 lost fragments, 18 were of *C. hystrix* origin, four were *C. sativus*-specific, and the remaining two were shared by both species, implying that fragment loss may be correlated with haploid DNA content (genome size) of diploid parents. Most changes were observed in the first generation after polyploidization (S_1) and stably inherited in the subsequent three generations (S_2–S_4), indicating that genomic changes might be a rapid driving force for the stabilization of allotetraploids. Sequence analysis of 11 of the 28 altered DNA fragments showed that genomic changes in the allotetraploid occurred in both coding and non-coding regions, which might suggest that retrotransposons inserted into genome randomly and had a genome-wide effect on the allotetraploid evolution. Fluorescence in situ hybridization (FISH) analysis revealed a unique distribution of retrotransposon and/or microsatellite flanking sequences in mitotic and meiotic chromosomes, where the preferential FISH signals occurred in the centromeric and telomeric regions, implying that these regions were the possible hotspots for genomic changes.

Keywords Polyploidization · Evolution · Retrotransposon · *Cucumis* · IRAP · REMAP · Fluorescence in situ hybridization (FISH)

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Introduction

Allopolyploidization is an evolutionary process whereby two or more different genomes are joined into the same nucleus by inter-specific or inter-generic hybridization followed by chromosome doubling (Feldman and Levy 2005). Allopolyploidization has played an important role in plant evolution (Leitch and Bennett 1997; Wendel 2000). Genome size measurements and comparative molecular analyses from previous studies have revealed that the polyploid genome is not a simple addition of diploid genomes from which they are derived (Dolezel et al. 1998; Leitch and Bennett 2004; Messing et al. 2004). Generally, the formation of allopolyploid is accompanied by rapid genetic and epigenetic changes, which contribute to stabilization of the newly formed species (Song et al. 1995; Feldman et al. 1997; Comai 2000; Ozkan et al. 2001; Madlung et al. 2002; Kashkush et al. 2002; Adams et al. 2003; Feldman and Levy 2005).

McClintock (1984) proposed that newly formed allopolyploids underwent a huge 'genomic shock', which could trigger the activation of retrotransposons. Many studies in different species were in support of this hypothesis (Liu and Wendel 2000; Kashkush et al. 2002, 2003; Liu et al. 2004; Madlung et al. 2005; Shan et al. 2005; Josefsson et al. 2006; Petit et al. 2010; Kraitshtein et al. 2010). However, these studies focusing on different allopolyploid species only provide evidence for the transcription of retrotransposons activated by allopolyploidization. Little is known about the impact of retrotransposons on allopolyploid evolution (Madlung et al. 2005; Beaulieu et al. 2009; Kraitshtein et al. 2010).

Since accurate genetic information of parental lines and derived allopolyploid can be obtained conveniently, newly synthesized allopolyploid serves as a unique model system for tracing the mobility of retrotransposons during allopolyploidization. *Cucumis* × *hytivus* Chen & Kirkbride ($2n = 4x = 38$) is a newly synthesized allotetraploid derived from interspecific hybridization from *C. sativus* L. ($2n = 2x = 14$) and *C. hystrix* Char. ($2n = 2x = 24$) and subsequent tissue culture-induced chromosome doubling (Chen et al. 1997; Chen and Kirkbride 2000). Our previous studies revealed that allopolyploidization in *Cucumis* induced extensive genome changes and cytological diploidization as well as epigenetic changes such as alterations of cytosine methylation, activation of retrotransposons, gene silencing and gene activation in early generations of this newly synthesized allotetraploid (Chen et al. 2007; Chen and Chen 2008; Zhuang and Chen 2009; Jiang et al. 2011).

Previous studies focused mainly on genetic changes following polyploid formation; little is known about the distribution of these changes in the chromosomes, which may provide valuable information in understanding polyploid evolution and speciation. For example, in *Triticale*, Bento et al. (2008) used Fluorescence in situ hybridization (FISH) technique to reveal the chromosomal distribution of retrotransposon-microsatellite amplified polymorphism (REMAP) products (retrotransposon- and/or microsatellite-flanking sequences), and their result showed the preferential distribution of REMAP products in condensed subtelomeric domains, which implied the putative role of retrotransposons and/or microsatellite in the establishment of terminal heterochromatin. Therefore, the objectives of the present study were to investigate the effect of retrotransposons on genomic changes in early generations after synthesis of *C. hytivus* and to localize physically these changes in cucumber chromosomes. Two retrotransposon-based markers, inter-retrotransposon amplified polymorphism (IRAP), REMAP and the inter simple sequence repeat (ISSR) marker were utilized to study genomic changes of retrotransposon- and/or microsatellite-associated sequences in the first four generations of *C. hytivus* allotetraploid. Changed fragments were also identified and sequenced, which were then employed in FISH to locate retrotransposon- and/or microsatellite-flanking sequences in mitotic and meiotic chromosomes.

Materials and methods

Plant materials

Plant materials used in this study included two highly inbred diploid parental lines, the cultivated cucumber cultivar *C. sativus* cv. Beijingjietou ($2n = 2x = 14$, genome CC) and a wild relative *C. hystrix* ($2n = 2x = 24$, genome HH), and S_1–S_4 generations of a synthetic allotetraploid, *C. hytivus* ($2n = 4x = 38$, HHCC). The allotetraploid was obtained from interspecific hybridization, embryo rescue and chromosome doubling (Chen and Kirkbride 2000) with subsequent self-pollination to constitute the first four inbreeding generations (S_1–S_4).

DNA extraction

Genomic DNA was extracted from young leaves of seedlings using the cetyltrimethylammonium bromide method described by Murray and Thompson (1980). DNA quality was evaluated by electrophoresis in 1% agarose gel. DNA stocks were diluted to 50 ng/μl for subsequent uses.

IRAP procedure

The IRAP analysis followed Kalendar et al. (1999). Four primers were designed based on the long terminal repeat

Table 1 Primers used for PCR analysis to study the effect of retrotransposons on genomic changes during allopolyploid evolution in *Cucumis*

Primer	GenBank accession #	Primer sequence (5′–3′)
IRAP		
LTR1	GQ326556	TCGCTGAAGTGAATGGTGGTC
LTR2	GQ326556	CGATAATACAACCCTCCGAAG
LTR3	AM1174993	ATTCTTCTATCCTTGCCCGTGG
LTR4	AM1174993	GACATTTTGGGAGGGATTGGC
ISSR		
$(AC)_8GC$		ACACACACACACACACGC
$(AG)_8TG$		AGAGAGAGAGAGAGAGTG
$(GA)_9C$		GAGAGAGAGAGAGAGAGAC
$(CA)_9G$		CACACACACACACACACAG
$(CTC)_7G$		CTCCTCCTCCTCCTCCTCCTCG
REMAP		
REMAP1		LTR1/$(AC)_8GC$
REMAP2		LTR1/$(CA)_9G$
REMAP3		LTR1/$(CTC)_7G$
REMAP4		LTR2/$(AC)_8GC$
REMAP5		LTR2/$(GA)_9C$
REMAP6		LTR2/$(CTC)_7G$
REMAP7		LTR4/$(CA)_9G$

IRAP inter-retrotransposon amplified polymorphism, *REMAP* retrotransposon-microsatellite amplified polymorphism, *ISSR* inter-simple sequence repeat

(LTR) regions of two Ty1-*copia* retrotransposons: T7 (GQ326556) in *C. sativus* and *Reme* 1 (AM1174993) in *C. melo* L. (Table 1). The PCR reaction mixture of 20 μl consisted of 50 ng template DNA, 0.5 μM primer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1× reaction buffer and 1U *Taq* DNA polymerase (TaKaRa, Japan). The PCR program followed Kalendar et al. (1999) except that the annealing temperature was changed to 56°C.

Inter simple sequence repeat procedure

ISSR amplification was carried out on the same set of materials used in IRAP and REMAP analysis. Five ISSR primers used are shown in Table 1. All the amplification conditions were the same as for IRAP analysis.

REMAP procedure

The amplification procedure of REMAP was also performed according to the protocol described by Kalendar et al. (1999). LTR primers were combined with ISSR primers performing REMAP primer combinations (Table 1). The PCR reaction and program were similar to those of IRAP.

Electrophoresis and data analysis

PCR products were resolved in 2% agarose gels (BIO-WEST, Spain) in 1× TAE buffer for 2–3 h at 95 volts, stained with ethidium bromide, and then photographed with an electrophoresis image analysis system (Peiqing, China). Only bands with sizes between 100 and 2,000 bp were scored because of high resolution obtained in this region. Quantitative differences between bands and minor non-reproducible bands were not considered. The presence or absence of each single fragment was scored as 1 or 0, respectively.

Cloning and analysis of DNA sequence

DNA fragments with altered patterns between parental lines and the allotetraploid (parental bands absent in the allotetraploid or novel bands in the allotetraploid) were excised from the gel, purified with a DNA gel extraction kit (Karroten, China), cloned into the pMD19-T vector (TaKaRa, Japan), and transformed into *E. coli* strain DH5α. Recombinant clones of expected sizes were sequenced at Invitrogen Bio-Technology Co., Ltd., (Shanghai, China). The sequences were used for BLAST against the National Center Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/BLAST/), the cucurbit genomic database (http://www.icugi.org/) and cucumber genome database (http://www.cucumber.genomics.org.cn/).

Fluorescence in situ hybridization (FISH)

DNA fragments from REMAP analysis with primer combination LTR4 and $(CA)_9G$ were utilized as probes for FISH in *C. sativus* with 45S rDNA sequence (close to centromere region) as the control. Mitotic metaphase chromosomes and meiotic pachytene chromosomes were prepared from root-tips and young flower buds of *C. sativus*, respectively, according to Han et al. (2008). FISH procedure followed Jiang et al. (1995). The samples were labeled with either biotin-16-dUTP or digoxigenin-11-dUTP and detected with fluorescein isothiocyanate-conjugated anti-biotin antibody and rhodamine-conjugated anti-digoxigenin antibody (Roche), respectively. Chromosomes were counterstained with DAPI in an anti-fade solution VectorShield (Vector Laboratories, Burlingame, CA), and images were captured using a SenSys CCD camera attached to an Olympus BX60 microscope. The CCD camera imagines were driven by the Applied Spectral Imaging FISHview 5.5 software (Applied Spectral Imaging, Inc., USA), and Photoshop software (Adobe Systems) was used to obtain optimal images.

Results

Genomic changes in early generations of *Cucumis* allotetraploid-*C. hytivus*

Four LTR primers were designed based on two retrotransposons, one from *C. sativus* (GQ326556) and another from *C. melo* (AM1174993) (Table 1). All four LTR primers and five anchored microsatellite primers were used in IRAP and ISSR analysis, respectively (Table 1). Furthermore, LTR primers were combined with ISSR primers for REMAP analysis. Only highly polymorphic primer combinations with good reproducibility were selected. Eventually, seven primer combinations were selected for REMAP analysis (Table 1).

Thirty-three and 66 bands in the size range of 200–2,000 bp were amplified from two diploid parents (*C. hystrix* and *C. sativus*) using IRAP and REMAP markers, respectively. IRAP produced 8.1 bands on average, whereas REMAP yielded 9.4 bands for each PCR reaction. The ISSR markers generated a total of 40 bands with an average of 8.0 bands per PCR reaction. The total number of bands and their distribution among the first four generations of *C. hytivus* as well as two diploid parents detected by these three molecular maker systems are shown in Table 2. The monomorphic band means the band that presents in all the materials while the polymorphic one just presents in some of the materials. The ratio of polymorphic bands obtained with IRAP, REMAP, and ISSR analyses was 57.6% (19/33), 59.1% (39/66), and 70% (28/40), respectively (Table 2).

To evaluate the frequency of genomic changes in *Cucumis* allotetraploid, the total number of bands of amplified from both diploid parents was compared with bands observed in *C. hytivus*. About 82.2% (111/135) of the parental fragments were conserved in *C. hytivus*. Of the primers used herein, 28 changed fragment (24 fragment loss and four novel bands) were detected in *C. hytivus* when compared to their diploid parents. The changes were manifested by loss of 18 *C. hystrix*—specific bands (64.3%), 4 *C. sativus*—specific bands (14.3%), and two shared by both parental species (7.1%), as well as appearance of four novel bands (14.3%), which were not seen in either of the parental species (Table 3; Fig. 1). The dynamics of genomic changes was also examined among the first four generations after synthesis of *Cucumis* allotetraploid. Nearly all the genomic changes occurred as early as the first generation. Notably, however, one parental

Table 2 Results of IRAP, REMAP and ISSR analysis in two parental lines *C. sativus* (CC), *C. hystrix* (HH) and their synthesized allotetraploid *C. hytivus* (S_1–S_4)

Markers	Genotypes[a]						Total number[b]
	HH	CC	S_1	S_2	S_3	S_4	
IRAP	+	+	+	+	+	+	14
	+	−	+	+	+	+	7
	−	+	+	+	+	+	4
	+	−	−	−	−	−	5
	−	+	−	−	−	−	1
	+	+	+	−	−	−	1
	−	−	+	+	+	+	1
Total							33
REMAP	+	+	+	+	+	+	27
	+	−	+	+	+	+	12
	−	+	+	+	+	+	14
	+	−	−	−	−	−	9
	−	+	−	−	−	−	2
	−	−	+	+	+	+	2
Total							66
ISSR	+	+	+	+	+	+	12
	+	−	+	+	+	+	12
	−	+	+	+	+	+	9
	+	−	−	−	−	−	4
	−	+	−	−	−	−	1
	+	+	−	−	−	−	1
	−	−	+	+	+	+	1
Total							40

[a] Fragments detected by these three markers. '+', fragment present; '−', fragment absent

[b] The number of fragment detected by these three markers

Table 3 Characterization of parental fragment lost in the allotetraploid *Cucumis hytivus*

Fragments in parent[a]			Total[b]	Fragment number disappeared in allotetraploid[c]		
Polymorphic						
HH (♀)	CC (♂)	Monomorphic		♀	♂	Total
49	31	55	135	18 + 2[d]	4 + 2[d]	24

[a] The fragments amplified in two diploid parents

[b] The total number of fragments amplified in both parents (*C. hystrix* and *C. sativus*). Monomorphic fragments were scored only once

[c] The fragments present in either diploid parent but disappeared in the allotetraploid (*C. hytivus*)

[d] The fragments existed in both parental species (*C. hystrix* and *C. sativus*) that were lost in *C. hytivus*

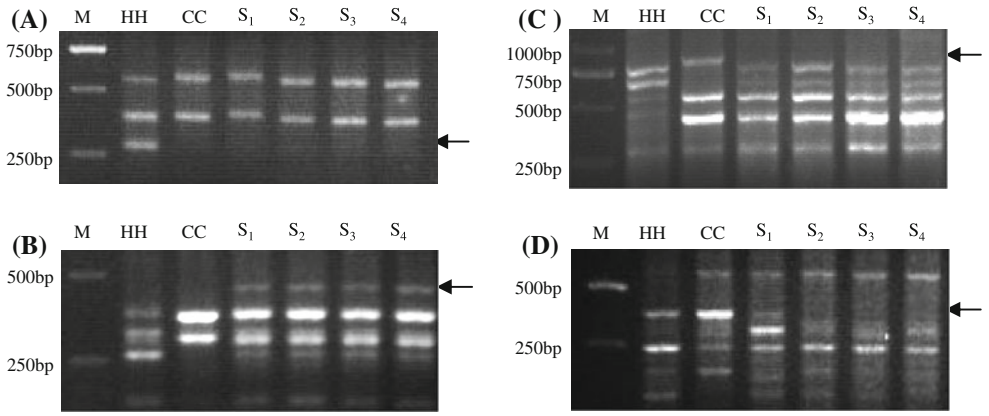

Fig. 1 Representative IRAP, REMAP and ISSR agarose gel profiles of first four generations of the synthetic allotetraploid *Cucumis hytivus* (S_1–S_4) and their diploid parents, *C. hystrix* (HH) and *C. sativus* (CC). **a** REMAP2 primer combination LTR1/$(CA)_9$G: A fragment from maternal parent was lost in allotetraploid. **b** ISSR primer $(GA)_9$C: A novel DNA fragment presented in all four generations (S_1–S_4) of the allotetraploid when compared to diploid parents. **c** IRAP primer LTR4: One fragment from paternal parent (CC) was lost in the allotetraploid. **d** IRAP primer LTR1: the parents' band was present in S_1, but lost in S_2–S_4 generations. The *arrows* point to differential fragments between the parents and the allotetraploid

fragment detected by IRAP maker was inherited in the first generation, and then disappeared in subsequent generations (S_2–S_4).

Characterization of altered genomic DNA sequences

The fragments that were altered during the formation of allotetraploid were recovered from the gel, purified, and sequenced. Ultimately, 11 sequences were obtained (Table 4). The DNA sequences of the 11 fragments are provided in supplemental file one. Among the 11 sequences, nine (R1–R9) presented in one or both parental lines were absent in the allotetraploid, and two novel fragments (R10–R11) were present in first four generations of allotetraploid. Sequence analysis (Table 4) showed that four (R3, R4, R8, and R11) possessed no similarity to any known gene. R1 was similar to *Gibberella zeae* PH-1 hypothetical protein. R5 and R6 had a high similarity to *C. sativus* T7 retrotransposon. Likewise, R2, R7, R9 and R10 showed high similarity to cucumber zinc ion binding protein, cucumber 3-hydroxyacyl-CoA dehydrogenase, *Agrobacterium tumefaciens* ABC transporter (a membrane spanning protein), and cucumber carbonate dehydratase, respectively.

Chromosomal distribution of retrotransposon and/or microsatellite flanking sequences

The FISH examination of meristematic root-tip cells as well as young flower buds revealed the chromosomal distribution of retrotransposon *Reme* 1 and microsatellite $(CA)_9$G flanking sequences (REMAP7 PCR products) in *C. sativus*. The strongest hybridization signals were located in the heterochromatic regions (intensively stained by DAPI) in interphase nucleus (Fig. 2a, b). The distribution of signals was even more obvious in mitotic metaphase chromosomes. Hybridization signals were present in all 14 *C. sativus* chromosomes with varying distribution patterns and signal intensities along different chromosomes (Fig. 2c, d). For example, REMAP7 displayed strong

Table 4 Molecular characterization of the recovered fragments that subject to genetic changes in *Cucumis hytivus* when compared with its diploid parents (*C. sativus* and *C. hystrix*)

Primer	Code[a]	The pattern of alteration fragments[b]						Sequence similarity[c]	E value
		HH	CC	S_1	S_2	S_3	S_4		
LTR3	R1	+	−	−	−	−	−	*Gibberella zeae* PH-1 hypothetical protein	1e−33
REMAP1	R2	+	−	−	−	−	−	Cucumber zinc ion binding protein	1e−104
REMAP2	R3	+	−	−	−	−	−	No	–
REMAP4	R4	+	−	−	−	−	−	No	–
LTR2	R5	−	+	−	−	−	−	*Cucumis sativus* T7 retrotransposon	1e−175
LTR2	R6	−	+	−	−	−	−	*Cucumis sativus* T7 retrotransposon	9e−120
LTR4	R7	−	+	−	−	−	−	Cucumber 3-hydroxyacyl-CoA dehydrogenase	2e−35
$(AG)_8TG$	R8	−	+	−	−	−	−	No	–
$(CA)_9G$	R9	+	+	−	−	−	−	ABC transporter, membrane spanning protein in *Agrobacterium tumefaciens*	1e−66
$(GA)_9C$	R10	−	−	+	+	+	+	Cucumber carbonate dehydratase	1e−115
REMAP5	R11	−	−	+	+	+	+	No	–

[a] R represents the recovered fragment

[b] +, transcript present; −, transcript absent

[c] The search performed using BLAST analysis package (http://www.blast.ncbi.nlm.nih.gov/), the cucurbit genomic database (http://www.icugi.org/) and cucumber genome database (http://www.cucumber.genomics.org.cn/), where similarity was considered as significant for E-values $<e^{-10}$ (E-value means that the expected value of a random variable is the integral of the random variable with respect to its probability measure)

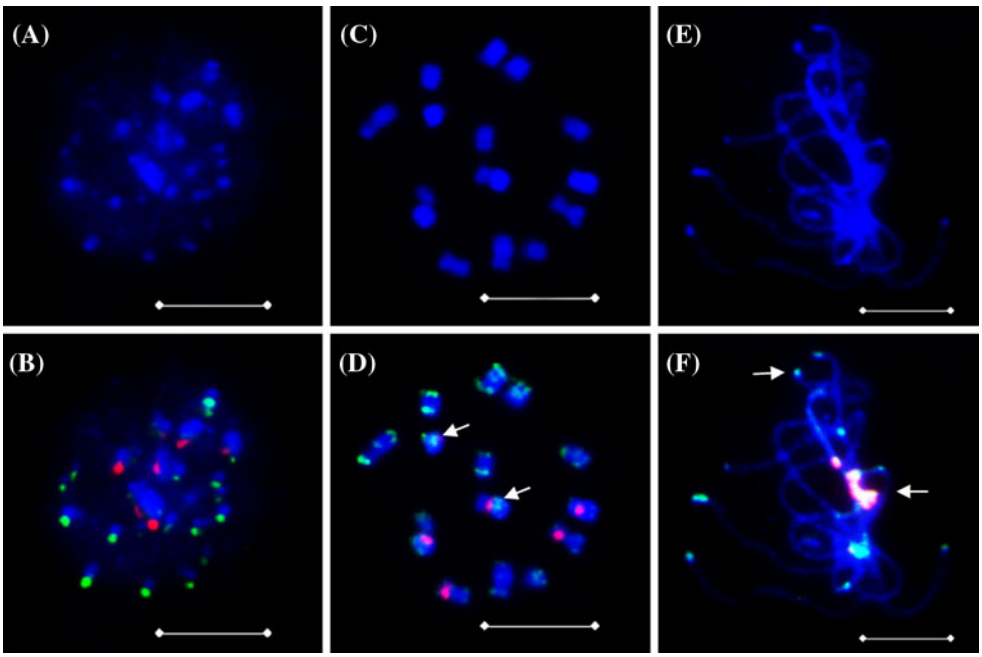

Fig. 2 Chromosomal location of flanking sequences of the *Reme* 1 retrotransposon and microsatellite $(CA)_9G$ (REMAP7 products) in *C. sativus* revealed by FISH. **a** Mitotic interphase nuclei counterstained with DAPI (*blue*). **b** Mitotic interphase nuclei probed with REMAP7 products (*green*) and 45S rDNA (*red*). **c** Mitotic metaphase chromosomes counterstained with DAPI (*blue*). **d** Mitotic metaphase chromosomes probed with REMAP products (*green*) and 45S rDNA (*red*). The *arrows* indicated the two chromosomes which had strong signals in the centrometric regions. **e** Meiotic pachytene chromosomes counterstained with DAPI (*blue*). **f** Meiotic pachytene chromosomes probed with REMAP7 products (*green*) and 45S rDNA (*red*). The *arrowheads and arrows* pointed at the centromeric and telomeric regions, respectively. *Bars* = 5 μm

hybridization signals at both distal ends of most chromosomes. Furthermore, nearly all the chromosomes showed weak signals in the centromeric regions, except two chromosomes which had intensive signals (Fig. 2d, indicated by arrows). A FISH analysis on meiosis pachytene chromosomes was performed to clarify the detailed distribution of signals (Fig. 2e, f). This assessment revealed that the majority of signals were in heterochromatic regions (DAPI blight), such as centromeric (arrowheads) and telomeric (arrows) regions (Fig. 2f).

Discussion

Allopolyploidization can be a revolutionary event in plants through which a new species is formed. This process can be accompanied with many changes in the genome (McClintock 1984) including activation of retrotransposons (Kashkush et al. 2002; Jiang et al. 2011). Once activated, retrotransposons will have significant influences on the newly formed allopolyploid. The significant role of retrotransposons on allopolyploid evolution was well demonstrated in two newly synthesized allopolyploids Triticale (Bento et al. 2008) and *Spartina anglica* (Baumel et al. 2002) using retrotransposon-based markers IRAP/REMAP, and microsatellite-based marker ISSR markers. In the present study, we employed these three markers to detect genetic changes associated with retrotransposons in early generations of the synthetic allotetraploid *Cucumis hytivus*, from two parental species *C. sativus* and *C. hystrix*. Extensive genetic changes were detected (Table 2; Fig. 1). Of the 135 bands scored in the two diploid parental species, 24 were lost but four novel bands were presented in the allotetraploids. The frequency of parental fragment loss was much higher than that of novel fragment gain, indicating that sequence loss may be a key event of genomic changes in *C. hytivus*. Moreover, these changes occurred in higher frequency than that estimated in a previously study (Chen et al. 2007). This was probably due to the fact that marker types used in the present study were based on retrotransposon sequences, which can be activated by allopolyploidization (Bento et al. 2008).

The successful novel allopolyploids are formed in one evolutionary step (Feldman and Levy 2005). However, the sequence variation events in triticale that followed chromosome doubling consisted of continuous modifications and lasted for the first five generations (Ma and Gustafson 2006). On contrast, most genomic changes detected herein occurred in the S_1 generation of *C. hytivus* after polyploidization. The rapid genomic changes in the synthetic allopolyploid following chromosome doubling may contribute to the successful establishment of the new species by providing an essential adjustment for the harmonious coexistence of the two diploid genomes in the same nucleus (Song et al. 1995; Ozkan et al. 2001; Chen et al. 2007).

Some studies suggested that sequence loss was a nonrandom and reproducible event in newly formed allopolyploids, which was independent of parental genotypes, their cytoplasm or the ploidy levels (Wendel 2000; Ozkan et al. 2001), but seemed to be related to the parental genome size, where preferential fragment loss was from the parent with a higher haploid DNA content (Bento et al. 2008, 2011). In synthetic octaploid triticale from hexaploid common wheat ($2n = 6x = 42$) and rye ($2n = 2x = 14$), Bento et al. (2008) found that sequence loss appeared to be related to the haploid DNA content of the diploid parents, in which, preferential fragment loss was from the parent with a higher haploid DNA content (rye, which has a greater haploid DNA content when compared to that of each genome complement from the hexaploid wheat). Our finding in the present study concurred with Bento et al. (2008), because the fragment loss in *C. hytivus* was mainly originated from *C. hystrix*, whose haploid DNA content is larger than that of *C. sativus* (unpublished data). The preferential loss of *C. hystrix* DNA sequences can reduce the discrepancy between two diploid genomes thus contributing stabilization of the new *C. hytivus* genome.

Previous studies indicated that DNA sequences subjected to genetic changes were from both coding and non-coding regions (Volkov et al. 1999; Contento et al. 2005; Chen et al. 2007). Our findings in the present study presented further support of the early notion. For example, the predicated putative functions of seven of the eleven sequences undergone genetic changes exhibited high similarity to different kind of genes, and the remaining four sequences didn't have coding function (Table 4). This observation may suggest that retrotransposons under investigation herein were inserted into the newly synthesized *C. hytivus* genome randomly, which further proved the genome-wide influence of retrotransposons.

Using FISH, we were able to study the distribution of retrotransposon and/or microsatellite flanking sequences on mitotic and meiotic chromosomes in *C. sativus* (Fig. 2). Hybridization signals in interphase nucleus were clustered in heterochromatic (DAPI bright) regions. In mitotic metaphase and meiotic pachytene chromosomes, FISH signals were concentrated on centromeric and telomeric regions. These results were similar to that observed by Bento et al. (2008) in triticale who found preferential disposition of REMAP products accumulated on terminal heterochromatin. The preferential distribution of retrotransposon and/or microsatellite flanking sequences on centromeric and telomeric regions may imply that these regions are the possible hotspots for genetic changes. Heterochromatin is rich in highly repetitive DNA

sequences, and plays an important role in the structural stability of the chromosomes (Dernburg et al. 1996; Karpen et al. 1996). Consequently, the damage of genetic changes occurred in the heterochromatin regions might be comparatively small, and could increase the differences between homoeologous chromosomes, which contributed to the normal chromosome pairing.

In conclusion, our results demonstrate that retrotransposons have a genome-wide effect on the newly synthesized *Cucumis* allotetraploid (*C. hytivus*) by triggering relatively rapid and preferential genomic changes. Such changes may be critical for the stabilization of allopolyploid genome, leading to the harmonious coexistence of the two different genomes (*C. sativus* and *C. hystrix*) in one nucleus of *C. hytivus*. Furthermore, it seems that these genomic changes occurred at non-random hotspot in the genome.

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