Genetic Variation Analysis of Watermelon Genomes with Different Ploidy

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Abstract 120 pairs of SSR primers and 63 pairs of InDel primers were used to determine genetic variation among diploid, triploid and tetraploid watermelon genomes. The results showed that 113 pairs of SSR primers and 63 pairs of InDel primers could be successfully amplified. There was slight difference between diploid and tetraploid watermelon genomes in the aspect of SSR. Among 120 pairs of SSR primers, 2 showed obvious polymorphism with polymorphic rate of 1.67%, corresponding to 34047313-34063581 intervals on chromosome 9. 1 gene encoding zinc finger protein was predicted in polymorphic interval which could regulate the expression of other genes at the level of transcription and translation. There was no significant difference in the aspect of InDel among watermelon genomes with different ploidy which indicated that the genomic insertion and deletion site was not changed before and after chromosome doubling. The results indicated that watermelon genomic structure had no significant change in the process of polyploidization. The difference of traits between tetraploidy and diploid parent might be closely related with epigenetic regulation. This study could provide scientific basis for ploidy breeding, new variety improvement and germplasm innovation.

Key words Watermelon, Diploid, Tetraploid, SSR, InDel

1 Introduction

Polyploidization, a form of plant's adaptation to environment, has a significant impact on species diversity[1]. About 70% of angiosperms in nature experience polyploidization in evolutionary history[2]. Due to increase in the number of chromosomes and gene interaction, there are changes in the chromosome structure and gene expression patterns, finally showing difference in morphological feature[3]. Polyploid plants have organs, significantly increased biomass and strong ability to adapt to the environment[4-5], often showing drought-resistant, cold-resistant and disease-resistant features, in line with the needs of agricultural production. Polyploid material has a very important value in the study of crop genetic improvement and new variety breeding, so it is of great importance to analyze the genetic variation of polyploid material[6]. As for the differences in plant diploid and tetraploid genome sequences, different researchers draw varying conclusions. Jiao Feng *et al.*[7] use RAPD markers to analyze the differences in mulberry diploid and tetraploid genomes, but do not find polymorphic bands. Wang Zhuowei *et al.*[8] use AFLP markers to compare the differences in mulberry diploid and tetraploid genomes, and results show that there are some changes in the genome sequence of them. Nie Lijuan *et al.*[9] use AFLP markers to detect changes in watermelon diploid and tetraploid genomes. Compared with RAPD and AFLP markers, SSR and InDel markers have advantages of simple operation, codominant inheritance, amplification stability and good reproducibility, so the result is more accurate and reliable. In recent years, as the watermelon genome sequencing and resequencing studies are carried out, it has provided favorable conditions for the development and application of watermelon SSR and InDel markers[10]. Up to now, the use of SSR and InDel markers to analyze the differences in watermelon genomes with different ploidy is rarely reported. In this study, with the watermelon of different ploidy as test material, we use SSR and InDel molecular marker technology to study the genetic variation of watermelon genomes with different ploidy, which is conducive to explaining the formation mechanism of polyploid superior agronomic traits, thereby providing a scientific basis for watermelon ploidy breeding, new variety improvement and germplasm innovation.

2 Materials and methods

2.1 Test materials The material was the elite inbred line of diploid watermelon FR-32-1B (2N) (round fruit, red flesh, medium size), bred by Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences after years of breeding. FR-32-1B (2N) was induced by oryzalin, and the homologous tetraploid material FR-32-1B (4N) was identified by root tip chromosome counting and flow cytometry ploidy detection. With FR-32-1B (4N) as female parent, FR-32-1B (2N) as male parent, the cross was made up to obtain the triploid material FR-32-1B (3N). The materials were sown in nutrition bowl.

2.2 Methods

2.2.1 Genomic DNA extraction. 10 plants were selected for each ploidy, and equal amount of young leaves were taken and

Received: August 17, 2016 Accepted: October 10, 2016
Supported by National Nonprofit Institute Research Grant of CATAS-TCGRI (1630032014019; 1630032015003).
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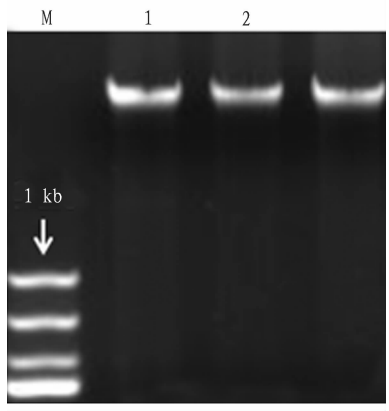
mixed for genomic DNA extraction. The CTAB method of Sharp *et al*.[11] was used to extract genomic DNA of diploid material FR-32-1B (2N), triploid material FR-32-1B (3N) and tetraploid material FR-32-1B (4N).

2.2.2 Analysis of polymorphic loci. Based on the developed watermelon SSR, InDel markers and established genetic linkage map[12], 120 pairs of SSR markers and 63 pairs of InDel markers evenly distributed on 11 watermelon chromosomes were selected. 25 pairs were on chromosome 1; 13 pairs were on chromosome 2; 16 pairs were on chromosome 3; 11 pairs were on chromosome 4; 22 pairs were on chromosome 5; 12 pairs were on chromosome 6; 14 pairs were on chromosome 7; 7 pairs were on chromosome 8; 27 pairs were on chromosome 9; 24 pairs were on chromosome 10; 12 pairs were on chromosome 11. The genomic DNA of FR-32-1B (2N), FR-32-1B (3N) and FR-32-1B (4N) was regarded as template to amplify and screen polymorphic SSR and InDel markers. PCR reaction system (10 μL) included 8 mmol L^{-1} Tris-HCl (pH 7.6), 50 mmol L^{-1} KCl, 1.0 mmol L^{-1} MgCl2, 0.20 mmol L^{-1} dNTPs, 50 ng primer, 0.60 U *Taq* DNA polymerase, and 50 ng template DNA. The amplification program: 94℃ initial denaturation for 5 min; 94℃ denaturation for 35 s; 55℃ annealing for 35 s; 72℃ extension for 1 min, 35 cycles; 72℃ final extension for 8 min. PCR product was stored at 10℃. 4 μL of amplification product was mixed with 2 μL of loading buffer solution for 4 h of 200 V electrophoresis by 12% non-denaturing polyacrylamide gel (acrylamide: bis-acrylamide = 29: 1), and the band was stained and analyzed.

2.2.3 Gene prediction and functional annotation in polymorphic interval. Using watermelon line 97103 genome database (http://www.icugi.org/cgi-bin/gb2/gbrowse/watermelon_v1/), the polymorphic interval was identified; using NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi), the functional annotation was conducted on the genes in the polymorphic interval.

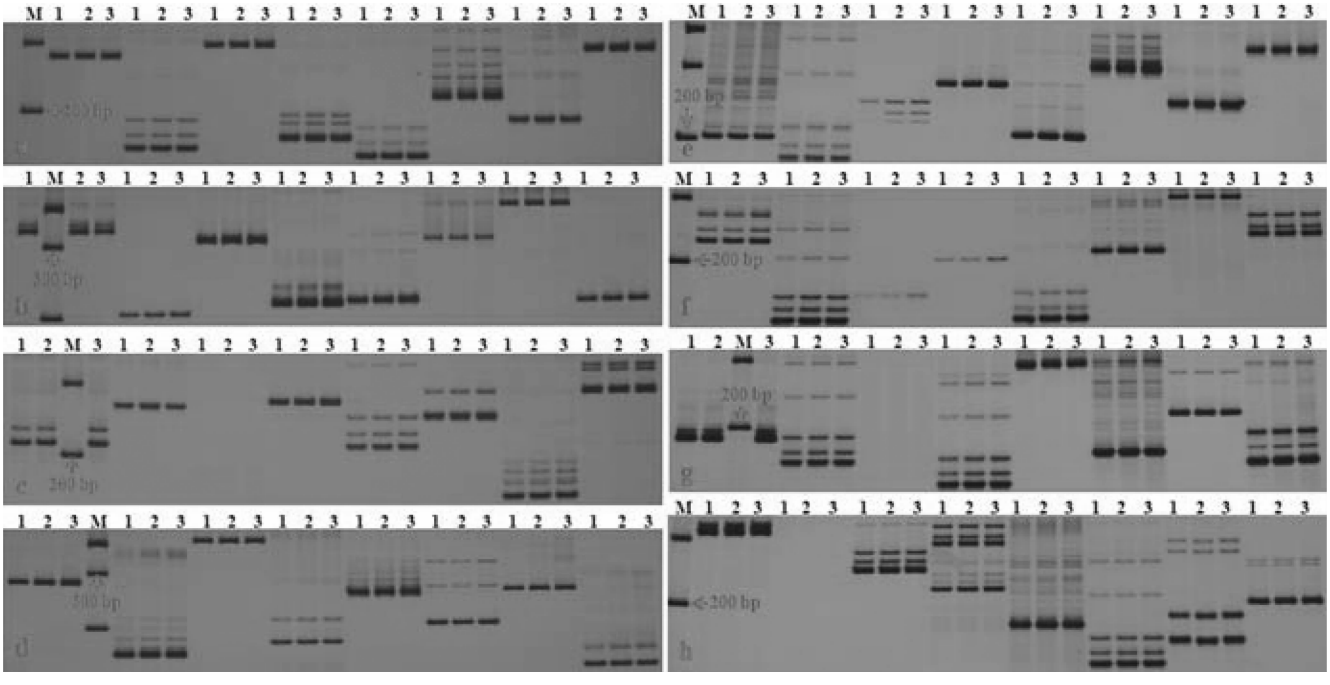
3 Results and analysis

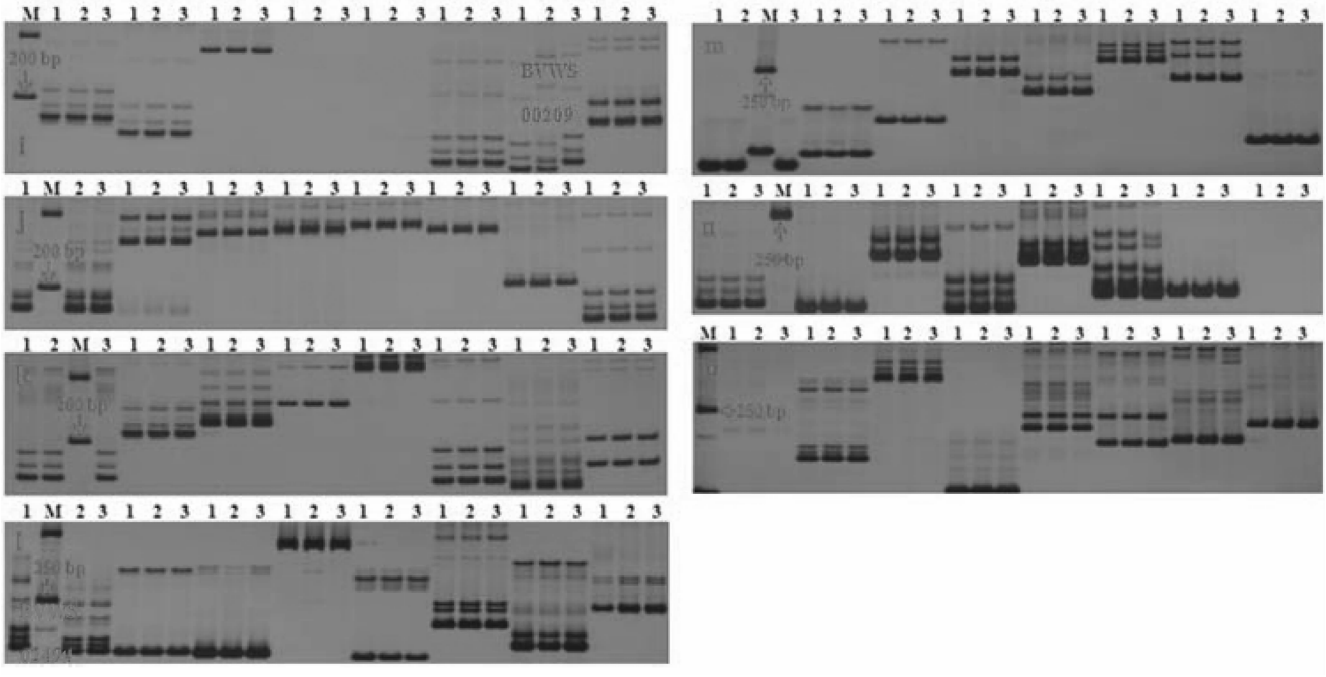
3.1 DNA extraction and detection Using modified CTAB method, the genomic DNA of watermelon with different ploidy was extracted, and the gel electrophoresis results showed that DNA bands were clear, having good integrity and high purity, without trailing and degrading effect. The ratio of A260 to A280 was 1.90, indicating that the extracted DNA was not contaminated by proteins, polysaccharides and RNA, fully meeting the requirements of the experiment.



M: DL1000 DNA Marker, 1: FR-32-1B (2N), 2: FR-32-1B (3N), 3: FR-32-1B (4N)

Fig. 1 Genomic DNA detection of watermelon with different ploidy



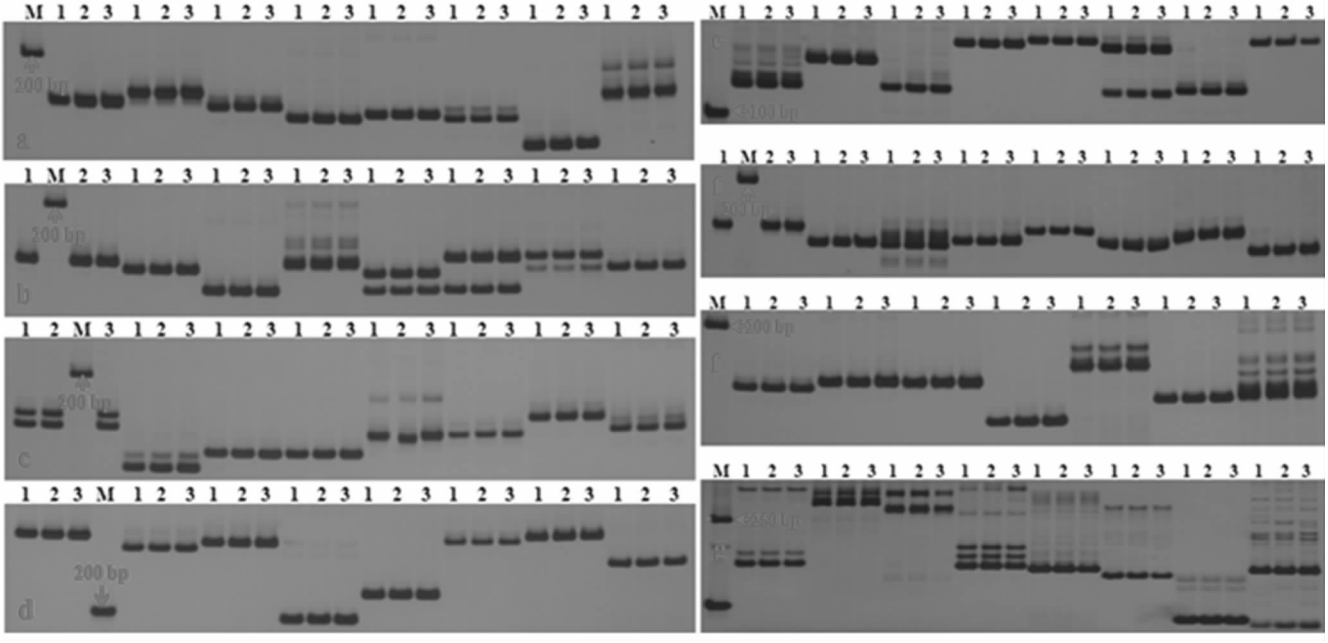


M:DL2000 DNA Marker,1:FR-32-1B (2N), 2:FR-32-1B (3N), 3:FR-32-1B (4N)

Fig. 2 Genomic SSR analysis of watermelon with different ploidy

Table 1 Polymorphic SSR primer sequence for different ploidy watermelon genomes

Name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
BVWS02494	TGCCGTTTGGATCACATAGA	CAATACGCACAAAAAGCGAA
BVWS00209	TGCTTCAAAATCTATTCACAATTTGC	TTCTTGGTTTCGGGTTTCTTTACA



M:DL2000 DNA Marker,1:FR-32-1B (2N), 2:FR-32-1B (3N), 3:FR-32-1B (4N)

Fig. 3 Genomic InDel analysis of watermelon with different ploidy

3.2 Genomic SSR analysis of watermelon with different ploidy With genomic DNA of watermelon with different ploidy as template, 120 pairs of SSR markers uniformly distributed on 11 watermelon chromosomes were selected for amplification, and 113 pairs of primers could amplify clear bands. The amplified band pattern of 111 pairs of primers was consistent for different ploidy watermelon materials, and the polymorphic fragments were not found, indicating that the 111 pairs of primers had the same bind-

ing site among the test materials, and the target region had the same number of repeating units. In diploid and tetraploid, 2 pairs of SSR markers (BVWS00209 and BVWS02494) showed significant polymorphism, accounting for 1.67% of all primers (Fig. 2, Table 1). These polymorphic loci may be related to chromosome breakage and repair, mitosis and meiotic mutation, transposon deletion and insertion, and gene translocation.

3.3 Genomic InDel analysis of watermelon with different ploidy With genomic DNA of different ploidy watermelon as template, 63 pairs of InDel markers evenly distributed on 11 watermelon chromosomes were selected to amplify, and the amplification results showed that there was no difference in amplified fragment size and number between different ploidy watermelon materials (Fig. 3). The results showed that there was no change in genomic insertion and deletion site before and after watermelon diploid doubling.

3.4 Gene functional annotation in the polymorphic interval The polymorphic SSR marker BVWS02494 was corresponding to the interval of watermelon chromosome 9 (34047313-34047470), and the polymorphic SSR marker BVWS00209 was corresponding to the interval of watermelon chromosome 9 (34063455-34063581). In the interval of 34047313-34063581, one gene was predicted, with gene code of Cla005553. This gene encoded a zinc finger protein, and it was likely to regulate gene expression at the transcriptional and translational level by the specific binding with target molecule DNA, RNA, DNA-RNA sequence, thereby participating in cell differentiation, embryonic development and other life processes.

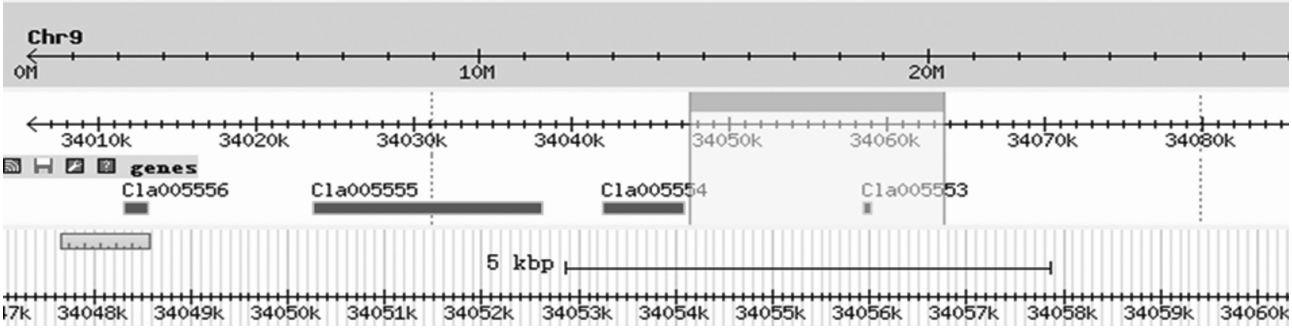


Fig. 4 Gene prediction in the polymorphic genomic region

4 Discussions

Polyploid breeding is an important area of plant genetics and breeding. Since the 1930s, China's polyploid application has been wide and there have been great social and economic benefits[13]. Polyploidization results in partial or complete duplication of genome. However, it is not the passive simple fusion of two genomes, and it involves a wide range of molecular and physiological adjustments. Numerous studies show that compared with diploid, there are varying degrees of variation in the expression pattern and level of polyploid genomes[14]. In the process of polyploidization of different species, there are differences in the genomic change level and manner. Compared with the diploid, there is slight variation in the polyploid genomes of cotton[15] and gorse[16], and the polyploid genomes of pomelo[17] undergo great loss after polyploidization. The genomes of "Zaohong 3" loquat also undergo significant correction after polyploidization[6]. The cause of this result may be related to the difference in mutagens, dosages and materials used by different researchers in induction of tetraploid. In this study, SSR and InDel markers were used to conduct genetic variation analysis on different ploidy watermelon genomes, and the results showed that there were also some differences in DNA level between watermelon tetraploid and diploid, indicating that in the induction of watermelon chromosome doubling, it caused changes in the nucleotide sequence of genomic DNA. The small changes in genomic sequence before and after watermelon chromosome doubling might be related to inadequate application of oryzalin in the induction of watermelon tetraploid. Plant polyploids often exhibit some morphological or physiological traits significantly different from the parental diploid. Then how are the traits of polyploid different from those of the parental diploid formed? Recent studies have found that it may be closely related to another regulation way (epigenetic regulation)[18]. Epigenetic regulation is the genetic regulation on gene expression without changing the gene sequence[19-20], including DNA methylation, histone modification and non-coding RNA regulation. And DNA methylation is a major epigenetic modification form[21]. Related studies show that the DNA methylation level of P. *fortunei* tetraploid is significantly higher than that of diploid, and DNA methylation may be one of the main reasons for the lack of doubling in morphological characters of tetraploid[22]. This conclusion has been verified in pear[23], *Eragrostis curvula*[24] and *Paspalum notatum*[25]. The next research step is to explore the mechanism of difference in traits between watermelon polyploid and diploid from the perspective of epigenetics.

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