Physical mapping of repetitive oligonucleotides facilitates the establishment of a genome map-based karyotype to identify chromosomal variations in peanut

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Abstract

Background: Chromosomal variants play important roles in crop breeding and genetic research. The development of single-stranded oligonucleotide (oligo) probes simplifies the process of fluorescence in situ hybridization (FISH) and facilitates chromosomal identification in many species. Genome sequencing provides rich resources for the development of oligo probes. However, little progress has been made in peanut. Thus, the identification of chromosomal variants in peanut remains a challenge, owing to a lack of efficient chromosomal markers.

Results: A total 114 new oligo probes were developed, based on the genome-wide tandem repeats (TRs) identified from the reference sequences of the peanut variety Tifrunner (AABB, 2n = 4x = 40) and the diploid species Arachis ipaensis (BB, 2n = 2x = 20). These oligos were classified into 28 types, based on their positions, and overlapping signals in chromosomes. For each oligo types, a single and representative oligos was selected and modified with 6-carboxyfluorescein (FAM) and 6-carboxytetramethylrhodamine (TAMRA). Based on these 28 probes, a new multiplex #3 cocktail was developed with FAM-modified TIF-439, TIF-185-1, TIF-134-3, and TIF-165-3, and TAMRA-modified Ipa-1162, Ipa-1137, DP-1, and DP-5. This cocktail enabled the establishment of a genome map-based karyotype after sequential FISH/genomic in situ hybridization (GISH) and in silico mapping. Furthermore, we identified 14 chromosomal variants of peanut induced by radiation. A total of 28 representative probes were further chromosomally mapped onto the new karyotype. Among the probes, eight were mapped in the secondary constrictions, and intercalary and terminal regions; four were B genome-specific; one was chromosome-specific; and the other 15 were extensively mapped in the pericentric regions of chromosomes.

Conclusions: The development of new oligo probes provides effective tools, which can be used to distinguish various chromosomes of peanut. Physical mapping reveals the genomic organization of repetitive oligos in peanut chromosomes by FISH. Following comparisons with their positions in the reference sequences, a genome map-based karyotype was established and used for the identification of chromosome variations in peanut.

Background

Chromosomal variations, such as translocations, inversions, duplications, and deletions play important roles in crop breeding and genetic research. Such variations are usually induced by physical [1-3], chemical [4, 5], and genetic factors [6]. A large number of chromosomal variants have been developed and used in tomato [1], wheat [4], corn [7], and cotton [8], among other crops.

Cultivated peanut (Arachis hypogaea L., 2n = 4x = 40, genome AABB) is a major oilseed and cash crop worldwide. In 2018, the total production of peanut worldwide was 45,950,900 Metric tons, with an average yield 1900.20 kg/ha [9]. Many gene mutants have been created and investigated [10-13]. Candidate genes controlling pod width [14], seed coat color [15], and semi-dwarf status [16] have been
identified by transcriptome sequencing of mutants. However, only a few chromosome variations have been reported due to the lack of detection methods and reference genomes [17].

The traditional methods of cytological analysis of chromosomal variations include chromosome C-banding [18], genomic in situ hybridization (GISH) [19], and fluorescence in situ hybridization (FISH) using plasmid clones of TRs [20-22] and transposons [23]. These methods have been used to establish karyotypes and to reveal both chromosomal structures and evolution. However, the karyotype resolution based on these methods is very low, and insufficient to identify chromosomal variants in peanut. Moreover, the time-consuming and expensive procedures associated with these methods limit their wide application and efficiency in practice.

The recently developed single-stranded oligonucleotide FISH (SSON FISH) provides a simple and efficient way to identify chromosomes in many species, including wheat [24, 25], rye [26], barley [27], maize [28], rice [29], potato [30], and cucumber [31]. The development of genome-wide TR oligos has considerably improved the resolution of karyotypes, which has facilitated the accurate identification of chromosomal variants [24, 32, 33]. Additionally, physical mapping of TRs not only reveals genome evolution, but also can guide sequence assembly in chromosomal regions with a high copy-number of repetitive sequences [24, 34].

In peanut, Du et al. [17] developed eight oligo probes, which they used in SSON FISH to karyotype different peanut varieties and to identify a chromosome translocation line. A more efficient oligo dye staining method was further developed and applied to a large number of peanut samples. However, because the oligos included only a limited number of TRs identified from the genome skimming of *A. duranensis*, the achieved karyotype resolution is still not high enough to identify more chromosomal variations. Therefore, it's necessary to mine the whole genome for TRs covering all chromosomes and chromosomal regions to improve the karyotype resolution of peanut.

Recently, the genome sequencing of peanut [36-38] and its wild donor species, *A. duranensis* and *A. ipaensis* [39], has been completed. This now provides high quality reference sequences to mine whole genome for representative TRs covering all chromosomes. In the reference genome map of peanut, the homologous chromosomes were numbered based on genetic linkage maps [36]. However, the allocated chromosome numbers in the references map do not correspond to the actual chromosomes in cytogenetic karyotypes [17-19] due to the lack of a link between genetic and cytogenetic maps.

This study aims to: 1) Mine oligos of whole genome for representative TRs based on the reference sequences of the peanut variety Tifrunner (AABB) [36] and *A. ipaensis* (BB) [39]. 2) Establish a genome map-based karyotype of Tifrunner and physically map repetitive oligos to reveal the genomic organization in peanut. 3) Use the karyotype to identify chromosomal variations induced by radiation.

**Results**

**Developing repetitive oligo probes from whole genome**
Genome sequences of Tifrunner and *A. ipaensis* were analyzed using the Tandem Repeats Finder (TRF) [40], resulting in 4,595 and 894 repetitive sequences, respectively. The length of these sequences varied between 4 bp and 723 bp, and the copy number between 50 and 29,162. After CD-HIT [41] elimination, a total of 80 and 35 TRs of Tifrunner and *A. ipaensis*, respectively, were selected for further development of oligos.

A total of 249 oligos were designed using the Oligo 7 [42], and *in silico* mapped to the reference sequences of peanut with B2DSC [32]. These oligos were first labeled via a random primer labeling method [28] and located on the chromosomes of peanut. 114 oligos produced clear signals in different positions of the chromosomes of Tifrunner (Table S1). Based on their unique patterns and positions after dual-color and sequential FISH, the 114 oligo probes were categorized into 28 types. Oligos with the same position and overlapping signals were classified as the same type. For each of the 28 types, a single oligo was selected and further modified with 6-carboxyfluorescein (FAM) or 6-carboxytetramethylrhodamine (TAMRA) (Table S2). Figure 1 showed the results of oligo Ipa-1463 after *in silico* mapping and FISH. 1,260 copies were observed in chromosome plots mapped in the region of 63–77 Mbp of chromosome B9 (Fig. 1a). The FISH analysis confirmed that the signals of this oligo were only present in one pair of chromosomes at a similar region. Thus, this chromosome actually corresponded with chromosome B9 in the reference sequence (Fig. 1b).

**Development of a genome map-based karyotype of Tifrunner**

Based on the unique patterns and sequence composition of 28 oligo probes, a new multiplex #3 oligo probe cocktail was developed with eight oligos, including FAM-modified TIF-439, TIF-185-1, TIF-134-3, and TIF-165-3, and TAMRA-modified Ipa-1162, Ipa-1137, DP-1, and DP-5. Both DP-1 and DP-5 were derived from a previous study by Du et al. [17]. Following the sequential FISH/GISH with multiplex #3, total genomic DNA of *A. duranensis* and *A. ipaensis*, and 45S and 5S rDNA assays, a robust karyotype of Tifrunner were established (Fig. 3a–e).

Comparisons of the distributions of the eight oligos in the karyotype and chromosome plots, we found that most signal sites and intensities in the actual chromosomes corresponded well with their positions and copy numbers in the reference sequences (Fig. S1). Finally, a genome map-based karyotype of Tifrunner was established. Each of the actual chromosomes in the karyotype were renumbered as A1~A10 and B1~B10, according to their pseudomolecule number in the genome map of Tifrunner [36]. However, in this karyotype, nine significant non-correspondent signals were observed in seven chromosomes (Fig. 3e–f). For example, the oligos Ipa-1162 and Ipa-1137 evidently had distribution sites on chromosomes A1 and B1 in the chromosome plots, but no signal was observed in the actual chromosomes. In contrast, oligos TIF-439, TIF-185-1, TIF-134-3, and TIF-165-3 produced strong signals in the centromeric regions of chromosomes B2 and B10, but were not *in silico* mapped in the chromosome plots (Fig. 3d–f, Fig. S1).

To validate the genome map-based karyotype, two chromosome-specific single-copy sequence oligo libraries, L1A-1 and L3A-1, from the upper arm of chromosomes A1 and A3, were used for sequential
FISH/GISH analysis, combined with the multiplex #3 and total genomic DNA of *A. duranensis* and *A. ipaensis* as probes (Fig. S2). The specific signals of the two chromosome-specific oligo libraries were clearly shown in the two expected chromosomes, indicating considerable correspondence between the actual chromosomes of the karyotype and the genome maps.

Among the A subgenome of this karyotype, chromosomes A1 and A8 (the smallest chromosome) both contained intense green signals in the centromeric regions, while A1 additionally had strong red signals in the terminal region of its short arm. Chromosomes A6, A7, and A10 had 45S or 5S rDNA sites. Chromosomes A2, A3, and A4 had green signals in the terminal regions of the short arms, while A3 and A4 had green signals in the centromeric regions. Chromosome A5 had red signals in the short arm, while A9 had red signals at the subtelomeric region of the long arm. Among the B genome, chromosomes B6, B7, and B8 showed signals with the probes for 45S or 5S rDNA. Chromosome B9 had red signals, and B3 had green signals in the centromeric regions. The other chromosomes had green signals either in the centromeric or telomeric regions with varying intensity (Fig. 3e). Based on the unique patterns observed, all chromosomes could be clearly differentiated in the karyotype.

**Chromosome allocation of the oligo probe**

To map TRs in the genome map-based karyotype of Tifrunner, 28 representative oligos were analyzed by both FISH and *in silico* mapping (Fig. 4). The 28 oligos produced more signals in chromosomes of the B genome than in those of the A genome (Fig. 4). Among them, six oligos (TIF-165-3, TIF-439, TIF-556, TIF-198-1, TIF-384-3, and TIF-185-1) produced signals in the interstitial or terminal regions of the chromosomes. Four oligos (TIF-198-2, TIF-416-3, TIF-497, and TIF-342-2) had signals exclusively on the B genome, indicating that these oligos are specific for the B genome. Two oligos (Ipa-1137 and Ipa-1162) had signals only at the secondary constrictions, which fully overlapped with the signals of 45S rDNA, following sequential FISH. Oligo Ipa-1463 exclusively showed signals on one pair of chromosomes, which indicated that it is chromosome-specific. The other 15 oligos had signals in the pericentric regions (Fig. S3).

Among the 28 oligos physically mapped via FISH, the distributions of 22 oligos were same or similar to those in the *in silico* mapping results. However, six oligos (TIF-89-3, TIF-155-5, TIF-198-1, TIF-359-3, TIF-76-1, and Ipa-1757) showed significant differences between the two maps. For example, TIF-89-3 and TIF-155-5 were *in silico* mapped in just two pairs of chromosomes with a high number of copies. However, eight pairs of chromosomes evidently showed FISH signals. Similarly, TIF-198-1 was *in silico* mapped onto one pair of chromosomes alone, but produced signals on 16 pairs of chromosomes. In contrast, obvious sites of TIF-76-1 were mapped to 13 pairs of chromosomes, but produced signals on only five pairs of chromosomes following FISH (Fig. 4, Fig. S4). This may indicated that not all TRs were unambiguously assembled in all chromosomes of Tifrunner.

**Identification of chromosomal variations of Silihong (SLH) induced by radiation**
To check chromosomal variations in peanut, sequential FISH using multiplex #3 assays, and GISH were conducted on Chinese variety SLH and 70 radiation-induced M₁ plants of SLH (Fig. 5). Fourteen M₁ plants showed chromosomal variations. For example, in plant 161-1a, one reciprocal translocation was evident, based on unique patterns, which probably occurred between chromosomes 1A and 3B. The segment of one chromosome translocation from the A genome had extensive green signals covering the arm and red signals at the terminal. This pattern was similar to that of the signals on the centromere and the lower arm (L) of 1A, and the signal patterns of another segment from the B genome was like the upper arm (S) of 3B with the centromere, which indicated that they were T 3BL-1AS·1AL and T 1AS·3BL-3BS. Further FISH was performed using the single-copy oligo library probes L1A-1 and L3A-1 which are exclusively hybridize to the upper arms of 1A and 3A. FISH signals confirmed the identification of the two translocated chromosomes (Fig. 5f–h).

A total of 13 other plants were identified with 17 translocations, one deletion, and eight monosomic chromosomes (Fig. S5). Among these chromosomal variations, eight translocations were observed between homoeologous chromosomes, and nine translocations were observed between non-homologous chromosomes. Chromosomes 1, 3, and 5 showed a greater number of translocations (Fig. 6).

Discussion

Applications of the new oligo probes

The TRs comprise a large proportion of the plant genome. In the past, DNA repeats were regarded as genomic 'junk' [43]. However, various studies in recent years have shown that TRs could be used to gain a better understanding of evolutionary history and genomic composition [44]. TRs also serve as placeholders for epigenetic signals that govern heterochromatin formation, and may have function in repairing double-strand DNA breaks [44, 45].

Many repetitive DNA elements generate specific distribution patterns on chromosomes among various species or genomes. These elements are the most common sources of probes for chromosome identification and cytogenetic studies in plants [24, 46, 47]. Du et al. [17] developed eight oligo probes of peanut, based on genome skimming of *A. duranensis*, and established a relatively high-resolution karyotype, which revealed various relationships among eight *Arachis* species. However, these oligo probes were neither genome-specific nor chromosome-specific, and did not cover the interstitial regions of peanut chromosomes.

In the present study, 114 new oligos covering different regions of peanut chromosomes were developed. 28 oligos, representing the 28 observed chromosome distribution patterns, were selected and mapped on the chromosomes. Eight oligos could be located in the secondary constrictions, interstitial, or terminal regions; four were B genome-specific, and one was chromosome-specific. These findings indicate that these probes will be useful for the precise identification of peanut chromosome translocations with a considerable high resolution, if various combinations of oligo probes are used. In comparison with
previously reported markers, such as retrotransposons [48], 45S and 5S rDNA plasmid clones [49], and a few bacterial artificial chromosome (BAC) clones [22], the 28 newly identified oligos produced signals covering different regions of chromosomes. Additionally, the development of the multiplex #3 assay will considerably simplify the FISH procedure. These new developments will facilitate chromosome engineering of peanut and support the development of further applications in the future.

Since repetitive sequences are likely evolved under different evolutionary pressures, comparative mapping of the repetitive sequences via FISH could facilitate phylogenetic analysis [34]. Based on repetitive sequences, the genomic formation of peanut as a tetraploid species has been supposed [22] and relationships among the A, B, K, F, E, and H genomes of 51 *Arachis* species have been described [35]. In addition, the 28 oligos identified in the present study also provide effective tools for genome evolutionary research of peanut and its relatives.

**Genome map-based karyotype of Tifrunner revealed specific characteristics in the distribution of repeated sequences in peanut**

Repetitive sequences in Tifrunner account for 74% of the assembled genome [36]. The recent event of sequencing the peanut genome [36-39] and numerous developments in peanut chromosome research [20-23], provided valuable information but the link between the reference genome map and the chromosome karyotype is still missing. Using the reference sequences of Tifrunner, oligos were *in silico* mapped and compared with the resulting FISH karyotype. Most of the repeat sequence positions and copy numbers in the reference map were the same or similar to their FISH signal distributions and intensities in the karyotype. Finally, a genome map-based karyotype of Tifrunner was established, in which all chromosomes were renumbered as indicated in the reference genome map.

Furthermore, our findings revealed that several repeats were inconsistently mapped in comparison to the actual chromosomes and the sequence map. For example, TIF-89-3, TIF-155-5, and TIF-198-1 produced FISH signals in more chromosomes than expected, while TIF-76-1 was *in silico* mapped on more chromosomes than the actual chromosomes with FISH signals. This indicated that the assembly of the reference sequences of Tifrunner still may need correction, particularly in regions with a high copy number of TRs. Ambiguity in the assembly of high-copy elements is a common difficulty in many species with large genomes. The present work provides visible evidence for further sequencing and assembly of the peanut genome.

**Importance of the identification of chromosomal variants for peanut research and breeding**

Development and physical mapping of the newly developed 28 oligos has considerably improved the karyotype resolution of peanut, which facilitates a more efficient identification of chromosomal variations. In the present study, 14 out of 70 radiation-induced M$_1$ plants were identified with translocations, deletions, and monosomic chromosomes. This indicates the potential of both the oligos identified and radiation in peanut chromosome engineering. The chromosomal variations could be used
for translocation or deletion mapping, radiation hybrid mapping, and gene mapping of peanut, as has been reported previously [50,51].

Furthermore, translocations occurring between partially homologous chromosomes or heterologous chromosomes promote the exchange of DNA beyond homologous chromosomes in conventional breeding, which could lead to new beneficial traits. In future studies, we intend to identify more translocations and produce inbred translocation lines to create homozygous translocations with beneficial genes. This should facilitate the genetic improvement of peanut.

Conclusions

Based on the reference sequences of Tifrunner and *A. ipaensis*, 114 new repetitive sequence oligos were developed and chromosomally positioned. They were classified into 28 types, mainly based on their positions and overlapping signals in chromosomes. A total of 28 individual oligos, representing the 28 types, were selected and modified with FAM and TAMRA. Following comparisons of the distributions of eight oligos on chromosome plots in the reference sequences of Tifrunner, a genome map-based FISH karyotype was constructed, in which all chromosomes were renumbered as the references. The present karyotype has considerably improved resolution and facilitates the identification of 14 chromosomal variations of SLH. However, several TRs produced signals that were inconsistent with their copy numbers and positions in the reference sequences of Tifrunner. This indicated that the assembly of the reference sequences of Tifrunner still needs correction. The unique distribution patterns of 28 oligos in the karyotype provide visible evidences for correction of such minor errors. Therefore, the present novel probes and karyotype provide effective tools for chromosome engineering and evolutionary studies of peanut.

Methods

Plant materials

Peanut (*A. hypogaea* L., 2n = 4x = 40) varieties, Tifrunner and SLH, were kept by Henan Academy of Crop Molecular Breeding, Henan Academy of Agricultural Sciences, China. To develop chromosomal variations, SLH plants at the flowering stage were irradiated with a dosage of 16 Gy of gamma ray using a $^{60}$Co source (Isotope Institute Co. Ltd., Henan Academy of Sciences). A total of 70 $M_1$ plants were used for this study.

Chromosome preparation

Chromosomes were prepared, as described by Du et al. [52] with minor adjustments. When the roots of the peanut plant reached about 2 cm long, root tips were cut and placed in 8-hydroxyquinoline for 3 h at 25 °C. They were then treated with nitrous oxide ($N_2O$) at a pressure of 0.8–1.2 MPa for 1.5 h, and fixed in absolute ethanol-glacial acetic acid (3:1) solution for 12 h at 25 °C. The treated root tips were then stored in a refrigerator at -20 °C until further use. The apex was resected and disintegrated in 45% glacial acetic
acid for 2–3 min, compressed, and frozen at -80 °C in an ultra-low temperature refrigerator for 24 h, and then left uncovered. The root material was dehydrated in absolute ethanol for 12 h and air-dried for FISH.

**Design of oligo probes**

The whole genome assembly sequences of Tifrunner and *A. ipaensis* were downloaded from PeanutBase (https://peanutbase.org/home). The sequences of both Tifrunner and *A. ipaensis* genomes were analyzed to obtain tandem repeat sequences using the Tandem Repeats Finder (TRF, version 4.09) [40], based on the methods of Tang et al. [32]. The following parameter was applied: match = 2; mismatch = 7; indel = 7; probability of match = 80; probability of indel = 10; min score = 50; and max period = 2000. Data with a period size > 4 and DNA copy number > 50 were obtained using a Python script. Tandem repeat sequences with > 75% identity were determined using the CD-HIT [41] for clustering and maintaining consensus for each cluster. Oligos with a length of 40–45 nt were then designed, based on the tandem repeat sequences and Oligo 7 [42].

To determine the effectiveness of each designed probe, oligos were first physically mapped onto reference sequences of the chromosomes. The sequence of every derived oligo probe was aligned using the Nucleotide Basic Local Alignment Search Tool (blastn) in the B2DSC2 server against the Tifrunner genome. The blast results were filtered using pident = 90% and qcovhsp = 90%. The physical location information was drawn using the Plot tool in B2DSC. Oligos with a rich distribution of sites were synthesized by the General Biosystems Company (Anhui, China).

**FISH and sequential FISH analysis**

Oligo probes were first labeled with biotin-16-dUTP or digoxigenin-11-dUTP, using end labeling techniques. The 10-µl system included: 2 µl 5 × terminal deoxynucleotidyl transferase (TDT) buffer; 0.5 µl dATP; 3.5 µl dd H₂O; 2 µl CoCl₂; 0.5 µl TDT enzyme; 0.5 µl biotin-16-dUTP or digoxigenin-11-dUTP; and oligo (1ng/µl) 1µl; maintained at 37 °C for 15 min. After screening, 28 oligo probes were obtained (Table S2). All repetitive oligos were modified at the 5′-ends with TAMRA or FAM by General Biosystems Company (Anhui, China) for further use in FISH. To establish the karyotype, a multiplex probe cocktail, named Multiplex #3, was developed. Multiplex #3 included two different groups of oligos (4-4) mix probes and labeled (FAM-modified TIF-439, TIF-185-1, TIF-134-3, and TIF-165-3; and TAMRA-modified Ipa-1162, Ipa-1137, DP-1, and DP-5).

Two single-copy oligo libraries (L1A-1 and L3A-1) from chromosomes A1 and A3 were developed using genomic sequences of *A. duranensis* in PeanutBase, according to the methods described by Du et al. [17]. Each single-copy oligo library was derived from the distal region of each chromosome. Libraries were synthesized by MYcroarray (Ann Arbor, MI, USA). The resulting libraries were amplified and labeled with biotin-16-dUTP or digoxigenin-11-dUTP, according to the MYcroarray_MYtags labeling protocol.

The FISH and sequential FISH procedures followed those described by Du et al. [17]. Briefly, the slides were denatured in 70% formamide at 75 °C for 70 s. The hybridization solution, including 3 µl of each
probe, was denatured for 13 min. The slides were immersed in the hybridization solution at 37 °C in a wet box for at least 12 h, and then washed 10 times with 2 × saline-sodium citrate at 42 °C. Slides were then stained with 4′, 6-diamidino-2-phenylindole (DAPI) and mounted with Mounting Medium.

Sequential FISH was performed to map the signals of oligo probes and correlate a sequenced chromosome with a cytologically identified chromosome. Thereafter, FISH was conducted using repetitive or single-copy oligo probes, which were then washed to remove all signals, and subsequently dried. The GISH procedure was performed using total genomic DNA of *A. duranensis* and *A. ipaensis* as probes, and FISH was conducted using 45S rDNA and 5S rDNA as probes.

**Capture of images and analysis**

The FISH images were captured using a Leica DM6000 fluorescence microscope (Leica) with a cooled charge-coupled device camera (Leica). Images were optimized for contrast and brightness using Adobe Photoshop. Approximately 3–5 cells of each accession were observed for karyotyping and chromosome diversity analysis. Most karyotypes were developed from a single cell unless chromosomes showed overlapping.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**

All the data pertaining to the present study have been included in table and/or figure form in the manuscript and authors are pleased to share analyzed/raw data and plant materials upon reasonable request. Other datasets supporting the conclusions of this article are included within the article and its additional files.

**Competing interests**

The authors declare that they have no competing interests.

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Authors’ contributions

XZ, PD, BH, and WD designed the experiments; LF, PD, QW, LL, TL, SW, JG, ZS, and SH performed the probe design and FISH, and analyzed the data; XZ and PD wrote the manuscript. All authors have read and approved the manuscript.

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Abbreviations

oligo: oligonucleotide; FISH: fluorescence in situ hybridization; TRs: tandem repeats; FAM: 6-carboxyfluorescein; TAMRA: 6-carboxytetramethylrhodamine; GISH: genomic in situ hybridization; SSON: single-stranded oligonucleotide; TRF: Tandem Repeats Finder; SLH: Silihong; BAC: bacterial artificial chromosomes; N₂O: nitrous oxide; TDT: terminal deoxynucleotidyl transferase; DAPI: 4’, 6-diamidino-2-phenylindole.

References

1. Bhala VP, Verma RC. Gamma rays induced chromosomal aberrations in tomato (Solanum lycopersicum L.). Chromosome Botany. 2018; 12(4):86-90.

2. Pu J, Wang Q, Shen Y, Zhuang L, Li C, Tan M, Bie T, Chu C, Qi Z. Physical mapping of chromosome 4J of Thinopyrum bessarabicum using gamma radiation-induced aberrations. Theor Appl Genet. 2015; 128(7):1319-1328.

3. Zhang J, Jiang Y, Guo Y, Li G, Yang Z, Xu D, Xuan P. Identification of novel chromosomal aberrations induced by 60Co-γ-irradiation in wheat-Dasypyrum villosum lines. Int J Mol. 2015; 16(12):29787-29796.

4. Ma X, Wang Q, Wang Y, Ma J, Wu N, Ni S, Luo T, Zhuang L, Chu C, Cho S, Tsujimoto H, Qi Z. Chromosome aberrations induced by zebularine in triticale. Genome. 2016; 59(7):485-492.

5. Xu T, Bian N, Wen M, Xiao J, Yuan C, Cao A, Zhang S, Wang X, Wang H. Characterization of a common wheat (Triticum aestivum L.) high-tillering dwarf mutant. Theor Appl Genet. 2017; 130(3):483-494.

6. Danilova TV, Zhang G, Liu W, Friebe B, Gill BS. Homoeologous recombination-based transfer and molecular cytogenetic mapping of a wheat streak mosaic virus and Triticum mosaic virus resistance gene Wsm3 from Thinopyrum intermedium to wheat. Theor Appl Genet. 2017; 130(3):549-556.
7. Kynast RG, Okagaki RJ, Galatowitsch MW, Granath SR, Jacobs MS, Stec AO, Rines HW, Phillips RL. Dissecting the maize genome by using chromosome addition and radiation hybrid lines. Proc Natl Acad Sci USA. 2004; 101(26):9921-9926.

8. Gao W, Chen ZJ, John ZY, Raska D, Kohel RJ, Womack JE, Stelly DM. Wide-cross whole-genome radiation hybrid mapping of cotton (Gossypium hirsutum L.). Genetics. 2004; 167(3):1317-1329.

9. Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#data/QC. Accessed 20 Aug. 2020.

10. Mbaye G, Soumboundou M, Diouf L, Ndong B, Djiboune AR, Sy PM, Dieng SM, Diouf M, Diouf NN, Barry A. Evaluation of the effects of irradiation of peanut grain by a gamma-ray beam on culture. Open Journal of Biophysics. 2017; 7(3):94-100.

11. Hake AA, Shirasawa K, Yadawad A, Nayak SN, Mondal S, Badigannavar AM, Nadaf HL, Gowda M, Bhat RS. Identification of transposable element markers associated with yield and quality traits from an association panel of independent mutants in peanut (Arachis hypogaea L.). Euphytica. 2017; 213(12):283.

12. Joshi P, Jadhav MP, Shirasawa K, Yadawad A, Bhat RS. Foliar disease resistant and productive mutants from the introgression lines of peanut (Arachis hypogaea). Plant Breeding. 2020; 139(1):148-155.

13. Nadaf HL, Biradar K, Murthy G, Krishnaraj PU, Bhat RS, Pasha MA, Yerimani AS. Novel mutations in oleoyl-PC desaturase (ahFAD2B) identified from new high oleic mutants induced by gamma rays in peanut. Crop Sci. 2017; 57(5):2538-2546.

14. Wan L, Li B, Lei Y, Yan L, Ren X, Chen Y, Dai X, Jiang H, Zhang J, Guo W, Chen A, Liao B. Mutant transcriptome sequencing provides insights into pod development in peanut (Arachis hypogaea L.). Front Plant Sci. 2017; 8:1900.

15. Wan L, Li B, Pandey MK, Wu Y, Lei Y, Yan L, Dai X, Jiang H, Zhang J, Wei G, Varshney RK, Liao B. Transcriptome analysis of a new peanut seed coat mutant for the physiological regulatory mechanism involved in seed coat cracking and pigmentation. Front Plant Sci. 2016; 7:1941.

16. Guo F, Ma J, Hou L, Shi S, Sun J, Li G, Zhao C, Xia H, Zhao S, Wang X. Transcriptome profiling provides insights into molecular mechanism in Peanut semi-dwarf mutant. BMC Genomics. 2020; 21(1):1-16.

17. Du P, Li L, Liu H, Fu L, Qin L, Zhang Z, Cui C, Sun Z, Han S, Xu J, Dai X, Huang B, Dong W, Tang F, Zhuang L, Han Y, Qi Z, Zhang X. High-resolution chromosome painting with repetitive and single-copy oligonucleotides in Arachis species identifies structural rearrangements and genome differentiation. BMC Plant Biol. 2018; 18(1).

18. Pierozzi N I, Galgaro M. L, Lopes C R. Application of C-banding in two Arachis wild species, Arachis Pintoi Krapov. and W.C. Gregory and A. villosulicarpa Hoehne to mitotic chromosome analyses. Caryologia. 2001; 54(4):377-384.

19. Seijo G, Lavia GI, Fernández A, Krapovickas A, Ducasse DA, Bertioli DJ, Moscone EA. Genomic relationships between the cultivated peanut (Arachis hypogaea, Leguminosae) and its close relatives
revealed by double GISH. Am J Bot. 2007; 94(12).

20. Robledo G, Seijo G. Species relationships among the wild B genome of Arachis species (section Arachis) based on FISH mapping of rDNA loci and heterochromatin detection: a new proposal for genome arrangement. Theor Appl Genet. 2010; 121(6):1033-1046.

21. Bertioli DJ, Vidigal B, Nielen S, Ratnaparkhe MB, Lee TH, Leal-Bertioli SCM, Kim M, Guimarães PM, Seijo G, Schwarzacher T, Paterson AH, Heslop-Harrison P, Araujo ACG. The repetitive component of the A genome of peanut (Arachis hypogaea) and its role in remodelling intergenic sequence space since its evolutionary divergence from the B genome. Ann Bot-London. 2013; 112:545–559.

22. Zhang L, Yang X, Tian L, Chen L, Yu W. Identification of peanut (Arachis hypogaea) chromosomes using a fluorescence in situ hybridization system reveals multiple hybridization events during tetraploid peanut formation. New Phytol. 2016; 211(4):1424-1439.

23. Do Nascimento EFDM, Dos Santos BV, Marques LOC, Guimarães PM, Brasileiro ACM, Leal-Bertioli SCM, Bertioli DJ, Araujo ACG. The genome structure of Arachis hypogaea (Linnaeus, 1753) and an induced Arachis allotetraploid revealed by molecular cytogenetics. Comp Cytogen. 2018; 12(1):111-140.

24. Jiang J. Fluorescence in situ hybridization in plants: recent developments and future applications. Chromosome Res. 2019; 27(3):153-165.

25. Du P, Zhuang L, Wang Y, Yuan L, Wang Q, Wang D, Dawadondup, Tan L, Shen J, Xu H. Development of oligonucleotides and multiplex probes for quick and accurate identification of wheat and Thinopyrum bessarabicum chromosomes. Genome. 2017; 60(2):93-103.

26. Fu S, Chen L, Wang Y, Li M, Yang Z, Qiu L, Yan B, Ren Z, Tang Z. Oligonucleotide probes for ND-FISH analysis to identify rye and wheat chromosomes. Sci Rep. 2015; 5(1).

27. Tang S, Qiu L, Xiao Z, Fu S, Tang Z. New oligonucleotide probes for ND-FISH analysis to identify barley chromosomes and to investigate polymorphisms of wheat chromosomes. Genes-Basel. 2016; 7(12):118.

28. Zhu M, Du P, Zhuang L, Chu C, Zhao H, Qi Z: A simple and efficient non-denaturing FISH method for maize chromosome differentiation using single-strand oligonucleotide probes. Genome. 2017; 60(8):657-664.

29. Liu X, Sun S, Wu Y, Zhou Y, Gu S, Yu H, Yi C, Gu M, Jiang J, Liu B, Zhang T, Gong Z. Dual-color oligo-FISH can reveal chromosomal variations and evolution in Oryza species. Plant J. 2019; 101:112-121.

30. Braz GT, He L, Zhao H, Zhang T, Semrau K, Rouillard J, Torres GA, Jiang J. Comparative Oligo-FISH Mapping: An efficient and powerful methodology to reveal karyotypic and chromosomal evolution. Genetics. 2018; 208(2):513-523.

31. Han Y, Zhang T, Thammapichai P, Weng Y, Jiang J. Chromosome-specific painting in Cucumis species using bulked oligonucleotides. Genetics. 2015; 200(3):771-779.

32. Lang T, Li G, Wang H, Yu Z, Chen Q, Yang E, Fu S, Tang Z, Yang Z. Physical location of tandem repeats in the wheat genome and application for chromosome identification. Planta. 2018; 249, 663–675.
33. Tang S, Tang Z, Qiu L, Yang Z, Li G, Lang T, Zhu W, Zhang J, Fu S. Developing new oligo probes to distinguish specific chromosomal segments and the A, B, D genomes of wheat (Triticum aestivum L.) using ND-FISH. Front Plant Sci. 2018; 9:1104.

34. Jiang J, Gill BS. Current status and the future of fluorescence in situ hybridization (FISH) in plant genome research. Genome. 2006; 49(9):1057-1068.

35. Du P, Cui C, Liu H, Fu L, Li L, Dai X, Qin L, Wang S, Han S, Xu J, Liu B, Huang B, Tang F, Dong W, Qi Z, Zhang X. Development of an oligonucleotide dye solution facilitates high throughput and cost-efficient chromosome identification in peanut. Plant Methods. 2019; 15(1):69.

36. Bertioli DJ, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, Leal-Bertioli SCM, Ren L, Farmer AD, Pandey MK, Samoluk SS, Abernathy B, Agarwal G, Ballén-Taborda C, Cameron C, Campbell J, Chavarro C, Chitikineni A, Chu Y, Dash S, El Baidouri M, Guo B, Huang W, Kim KD, Korani W, Lanciano S, Lui CG, Mirouze M, Moretzsohn MC, Pham M, Shin JH, Shirasawa K, Sinharoy S, Sreedasyam A, Weeks NT, Zhang X, Zheng Z, Sun Z, Froenicke L, Aiden EL, Michelmore R, Varshney RK, Holbrook CC, Cannon EKS, Scheffler BE, Grimwood J, Ozias-Akins P, Cannon SB, Jackson SA, Schmutz J. The genome sequence of segmental allotetraploid peanut Arachis hypogaea. Nat Genet. 2019; 51(5):877-884.

37. Zhuang W, Chen H, Yang M, Wang J, Pandey MK, Zhang C, Chang W, Zhang L, Zhang X, Tang R, Garg V, Wang X, Tang H, Chow C, Wang J, Deng Y, Wang D, Khan AW, Yang Q, Cai T, Bajaj P, Wu K, Guo B, Zhang X, Li J, Liang F, Hu J, Liao B, Liu S, Chitikineni A, Yan H, Zheng Y, Shan S, Liu Q, Xie D, Wang Z, Khan SA, Ali N, Zhao C, Li X, Luo Z, Zhang S, Zhuang R, Peng Z, Wang S, Mamadou G, Zhuang Y, Zhao Z, Yu W, Xiong F, Quan W, Yuan M, Li Y, Zou H, Xia H, Zha L, Fan J, Yu J, Xie W, Yuan J, Chen K, Zhao S, Chu W, Chen Y, Sun P, Meng F, Zhuo T, Zhao Y, Li C, He G, Zhao Y, Wang C, Kavikishor PB, Pan R, Paterson AH, Wang X, Ming R, Varshney RK. The genome of cultivated peanut provides insight into legume karyotypes, polyploid evolution and crop domestication. Nat Genet. 2019; 51(5):865-876.

38. Chen X, Lu Q, Liu H, Zhang J, Hong Y, Lan H, Li H, Wang J, Liu H, Li S, Pandey MK, Zhang Z, Zhou G, Yu J, Zhang G, Yuan J, Li X, Wen S, Meng F, Yu S, Wang X, Siddique KHM, Liu Z, Paterson AH, Varshney RK, Liang X. Sequencing of cultivated peanut, Arachis hypogaea, yields insights into genome evolution and oil improvement. Mol Plant. 2019; 12(7):920-934.

39. Bertioli DJ, Cannon SB, Froenicke L, Huang G, Farmer AD, Cannon EKS, Liu X, Gao D, Clevenger J, Dash S, Ren L, Moretzsohn MC, Shirasawa K, Huang W, Vidigal B, Abernathy B, Chu Y, Niederhuth CE, Umale P, Araújo ACG, Kozik A, Do Kim K, Burow MD, Varshney RK, Wang X, Zhang X, Barkley N, Guimarães PM, Isobe S, Guo B, Liao B, Stalker HT, Schmitz RJ, Scheffler BE, Leal-Bertioli SCM, Xun X, Jackson SA, Michelmore R, Ozias-Akins P. The genome sequences of Arachis duranensis and Arachis ipaensis, the diploid ancestors of cultivated peanut. Nat Genet. 2016; 48(4):438.

40. Benson G. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res. 1999; 27(2):573-580.

41. Li W, Godzik A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics. 2006; 22(13):1658-1659.
42. Rychlik W. OLIGO 7 primer analysis software. Methods Mol Biol. 2007; 402:35.
43. Orgel LE, Crick FH. Selfish DNA: the ultimate parasite. Nature. 1980; 284(5757):604-607.
44. Gemayel R, Vinces MD, Legendre M, Verstrepen KJ. Variable tandem repeats accelerate evolution of coding and regulatory sequences. Annu Rev Genet. 2010; 44:445-477.
45. Gemayel R, Cho J, Boeynaems S, Verstrepen KJ. Beyond junk-variable tandem repeats as facilitators of rapid evolution of regulatory and coding sequences. Genes- Basel. 2012; 3(3):461-480.
46. Kato A, Lamb JC, Birchler JA. Chromosome painting using repetitive DNA sequences as probes for somatic chromosome identification in maize. Proc Natl Acad Sci USA. 2004; 101(37):13554-13559.
47. Huang X, Zhu M, Zhuang L, Zhang S, Wang J, Chen X, Wang D, Chen J, Bao Y, Guo J, Zhang J, Feng Y, Chu C, Du P, Qi Z, Wang H, Chen P. Structural chromosome rearrangements and polymorphisms identified in Chinese wheat cultivars by high-resolution multiplex oligonucleotide FISH. Theor Appl Genet. 2018; 131(9):1967-1986.
48. Nielen S, Campos-Fonseca F, Leal-Bertioli S, Guimarães P, Seijo G, Town C, Arrial R, Bertioli D. FIDEL-a retrovirus-like retrotransposon and its distinct evolutionary histories in the A-and B-genome components of cultivated peanut. Chromosome Res. 2010; 18(2):227-246.
49. Robledo G, Lavia GI, Seijo G. Species relations among wild Arachis species with the A genome as revealed by FISH mapping of rDNA loci and heterochromatin detection. Theor Appl Genet. 2009; 118(7):1295-1307.
50. Tiwari VK, Heesacker A, Riera-Lizarazu O, Gunn H, Wang S, Wang Y, Gu YQ, Paux E, Koo D, Kumar A, Luo M, Lazo G, Zemetra R, Akhunov E, Friebel B, Poland J, Gill BS, Kianian S, Leonard JM. A whole-genome, radiation hybrid mapping resource of hexaploid wheat. Plant J. 2016; 86(2):195-207.
51. Masoudi-Nejad A, Nasuda S, Bihoreau M, Waugh R, Endo TR. An alternative to radiation hybrid mapping for large-scale genome analysis in barley. Mol Genet Genomics. 2005; 274(6):589-594.
52. Du P, Li L, Zhang Z, Liu H, Qin L, Huang B, Dong W, Tang F, Qi Z, Zhang X. Chromosome painting of telomeric repeats reveals new evidence for genome evolution in peanut. J Integr Agr. 2016; 15(11):2488-2496.

**Tables**

**Table 1.** Probes used to construct karyotypes
| Probe names | Dye    | Concentration (ng/µL) | Volume (µL) |
|-------------|--------|-----------------------|-------------|
| TIF-439     | FAM    | 1                     | 1.5         |
| TIF-185-1   | FAM    | 1                     | 1.5         |
| TIF-134-3   | FAM    | 1                     | 1.5         |
| TIF-165-3   | FAM    | 1                     | 1.5         |
| DP-1        | TAMRA  | 1                     | 1.0         |
| DP-5        | TAMRA  | 1                     | 1.5         |
| Ipa-1162    | TAMRA  | 1                     | 2.0         |
| Ipa-1137    | TAMRA  | 1                     | 2.0         |

TAMRA, 6-carboxytetramethylrhodamine; FAM, 6-carboxyfluorescein.

**Figures**

![Figure 1](image)

**Figure 1**

Confirmation of the orientation of chromosome B9 of cultivar Tifrunner. The chromosome plot is drawn using the Plot tool in the B2DSC server. (a) The red lines on the chromosome plot indicate that the consensus sequence of Ipa-1463 hits the sequences in the 63–77 Mbp region on the chromosome B9. (b) The location of the Ipa-1463 signals on the chromosome B9. Scale bar: 10 µm
Figure 2

FISH of each probe on chromosomes of the cultivar Tifrunner based on fluorescence in situ hybridization (a) TIF-97-1 (red, signals); (b) TIF-108-3 (red, signals); (c) TIF-261-3 (green, signals); (d) TIF-134-3 (green, signals); (e) Ipa-1162 (red, signals); (f) TIF-556 (red, signals); (g) TIF-439 (green, signals); (h) TIF-384-3 (red, signals). Scale bar: 10 µm
Figure 3

Sequential fluorescence in situ hybridization/genomic in situ hybridization (FISH/GISH) karyotype and chromosome plots of the cultivar Tifrunner. (a) FISH using Multiplex #3; (b) GISH using genomic DNA of A. duranensis (green) and A. ipaensis (red); (c) FISH using 45S rDNA (red) and 5S rDNA (green) as probes; (d) Karyotypes corresponding to chromosomes in the sequencing map of Tifrunner; SSON indicates the FISH karyotype using Multiplex #3; GISH indicates the FISH karyotype using total genomic DNA of A.
duranensis (green) and A. ipaensis (red); 45S/5S indicates the FISH karyotype using 45S rDNA (red) and 5S rDNA (green); (e) Idiogram karyotype of Tifrunner; (f) chromosome plots of Tifrunner; blue arrows in (e) and (f) indicate the non-correspondent signals of oligos Ipa-1162 and Ipa-1137 between the karyotype and chromosome plots; green arrows in (e) and (f) indicate the non-correspondent signals of oligos TIF-439, TIF-185-1, TIF-134-3 and TIF-165-3 between the karyotype and chromosome plots; and red arrows in (e) and (f) indicate the non-correspondent signals of oligos DP-5 between the karyotype and chromosome plots. Scale bar: 10 µm
Figure 4

Karyotypes of all 28 oligos representing the 28 distribution patterns by sequential fluorescence in situ hybridization (FISH) using 45S rDNA (red), 5S rDNA (green), and A. duranensis (green) and A. ipaensis (red) total genomic DNA as probes of the cultivar Tifrunner. Scale bar: 10 µm

Figure 5

Chromosome variants detected in the radiation-induced M1 plant 161-1a of the peanut cultivar Silihong (SLH) after sequential fluorescence in situ hybridization/genomic in situ hybridization (FISH/GISH). (a–d) Results of sequential FISH/GISH in SLH (a–b) and M1 plant 161-1a (c–d), using Multiplex #3 (a and c); A. duranensis genomic DNA (b and d; green); A. ipaensis genomic DNA (b and d; red). (e) Karyotypes of SLH and 161-1a. (f–g) Sequential FISH/GISH using probe Libraries L1A-1 (green) and L3A-1 (red). (h) Translocated chromosomes in 161-1a. SSON indicates the FISH karyotype using Multiplex #3; GiSH indicates the FISH karyotype using total genomic DNA of A. duranensis (green) and A. ipaensis (red). Scale bar: 10 µm
Figure 6

Karyotypes and translocated chromosomes of radiation-induced M1 plants. SSONs show the FISH karyotype using SSON probes Multiplex #3; GISH show the FISH karyotype using total genomic DNA of A. duranensis (green) and A. ipaensis (red). Scale bar: 10 µm

Supplementary Files

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