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RESEARCH ARTICLE

Chromosome painting of telomeric repeats reveals new evidence for genome evolution in peanut

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Abstract

Interspecific hybridization is an important approach to improve cultivated peanut varieties. Cytological markers such as tandem repeats will facilitate alien gene introgression in peanut. Telomeric repeats have also been frequently used in chromosome research. Most plant telomeric repeats are $(TTTAGGG)_n$ that are mainly distributed at the chromosome ends, although interstitial telomeric repeats (ITRs) are also commonly identified. In this study, the telomeric repeat was chromosomally localized in 10 *Arachis* species through sequential GISH (genomic in situ hybridization) and FISH (fluorescence in situ hybridization) combined with 4',6-diamidino-2-phenylindole (DAPI) staining. Six ITRs were identified such as in the centromeric region of chromosome B^i5 in *Arachis ipaënsis*, pericentromeric regions of chromosomes A^s5 in *A. stenosperma*, $B^{ho}7$ in *A. hoehnei* and A^v5 in *A. villosa*, nucleolar organizer regions of chromosomes A^s3 in *A. stenosperma* and $A^{di}3$ in *A. diogoi*, subtelomeric regions of chromosomes $B^{ho}9$ in *A. hoehnei* and $A^{du}7$ in *A. duranensis*, and telomeric region of chromosome E^s7 in *A. stenophylla*. The distributions of the telomeric repeat, 5S rDNA, 45S rDNA and DAPI staining pattern provided not only ways of distinguishing different chromosomes, but also karyotypes with a higher resolution that could be used in evolutionary genome research. The distribution of telomeric repeats, 5S rDNA and 45S rDNA sites in this study, along with inversions detected on the long arms of chromosomes K^b10 and $B^{ho}10$, indicated frequent chromosomal rearrangements during evolution of *Arachis* species.

Keywords: *Arachis* species, inversion, interstitial telomeric repeats, karyotype

1. Introduction

The telomere is a specific DNA-protein structure with functions of regulating cell-senescence and carcinogenesis (Aubert and Lansdorp 2008). It also protects chromosomes from exonuclease digestion (Blackburn 1991). In both animals and plants, telomere repeats generally comprise tandemly repeated DNA sequences of $(TTAGGG)_n$ and $(TTTAGGG)_n$ (Burr *et al.* 1992; Fajkus *et al.* 2005).

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Telomeric repeats are generally presented at chromosome ends. Nevertheless, interstitial telomeric repeats (ITRs) occur in plants (Regad *et al.* 1994) and animals (Nergadze *et al.* 2004; Mattos *et al.* 2014; Scacchetti *et al.* 2015), including human (Park *et al.* 1992). In plants, ITRs are found at different chromosomes locations, including centromeric, rDNA, subtelomeric and telomeric regions (Gortner *et al.* 1998; Tek and Jiang 2004; Presting *et al.* 1996). Further analysis revealed that ITRs involved chromosome breakage, recombination and amplification (Lin and Yan 2008; Bolzan 2012).

Peanut (*Arachis hypogaea*, 2n=4*x*=40, genome AABB) is one of the most important food and forage crops in the world. Previous studies identified 80 annual and perennial species of *Arachis* in nine taxonomic sections, including *Arachis* (A, B and D), *Caulorrhizae* (C), *Erectoides* (E), *Extranervosae* (Ex), *Heteranthae* (H), *Procumbentes* (P), *Trierectoides* (Trie), *Triseminatae* (Tris) and *Rhizomatosae* (R) (Fernández and Krapovickas 1994; Krapovickas and Gregory 1994; Valls and Simpson 2005). However, the classification and phylogeny of many *Arachis* species are still in a state of flux. For example, FISH (fluorescence in situ hybridization) analysis using 45S and 5S rDNA and heterochromatin as probes led to re-designation of the genomes of *A. benensis* and *A. trinitensis* as F, those of *A. batizocoi*, *A. cruziana* and *A. krapovickasii* as K, and those of *A. decora*, *A. palustris* and *A. praecox* as G (Robledo and Seijo 2010; Silvestri *et al.* 2014). However, the P genome of *A. chiquitana* (Mallikarjuna 2005) was re-grouped into the A-genome due to its smallest “chromosome A” and similar patterns of 45S and 5S rDNA and heterochromatin as shown in other A-genome species (Robledo *et al.* 2009).

Telomeric repeats were identified through FISH in many species (Okazaki *et al.* 1993; Meyne *et al.* 1995). To our knowledge, the only ITR analysis using FISH analysis in peanut was conducted by Zhang (2013) in the species *A. hypogaea*, *A. ipaënsis*, and *A. duranensis*, with six ITRs being identified in the species *A. hypogaea* and *A. ipaënsis*, and none in *A. duranensis*. In the current study, ITRs from 10 wild *Arachis* species were analyzed by FISH using telomeric repeat probes combining with GISH (genomic *in situ* hybridization), 5S and 45S rDNA FISH and DAPI staining to: 1) characterize telomeric repeat distributions in *Arachis* species; 2) develop karyotypes with a higher resolution in these species; and 3) reveal new genomic evidence for evolution of *Arachis* species.

2. Materials and methods

2.1. Plant materials

Ten *Arachis* species, *A. hypogaea*, *A. ipaënsis*, *A. duranensis*, *A. chiquitana*, *A. stenosperma*, *A. diogoi*, *A. villosa*, *A. batizocoi*, *A. hoehnei* and *A. stenophylla,* were used in the study. The accession number, chromosome number and genome constitutions of the 10 *Arachis* species were provided in Table 1.

2.2. Chromosome preparation

Seeds of the 10 *Arachis* species were germinated on moist filter paper at 25°C for 7 d. Healthy lateral root tips were excised and pretreated with 2 mmol L^{-1} 8-hydroxyquinoline for 3 h at 25°C, and fixed in absolute ethanol (3):glacial acetic acid (1) for 12 h at 4°C. Then, 0.3–0.5 mm of root tips meristem was excised and squashed in 45% glacial acetic acid and freezed at –80°C for 12 h. Thus, the spread chromosomes were dehydrated in 100% ethanol and dried in air after the cover slips were removed.

2.3. Cytogenetic analysis

Total genomic DNA was extracted from young fresh leaves of *A. ipaënsis* (2n=2x=20, BB) (Wang *et al.* 2002). The

Table 1 Plant materials used in this study and their chromosome constitution

Species	Accession no.	Plant introduction (PI) no.	Chromosome no.	Genome (references)
Arachis chiquitana	36027	PI 476006	2n=2x=20	AA (Robledo *et al.* 2009)
A. stenosperma	410	PI 338280	2n=2x=20	AA (Robledo *et al.* 2009)
A. diogoi	10602	PI 276235	2n=2x=20	AA (Robledo *et al.* 2009)
A. villosa	22585	PI 298636	2n=2x=20	AA (Robledo *et al.* 2009)
A. duranensis	7988	PI 219823	2n=2x=20	AA (Robledo *et al.* 2009)
A. hypogaea	Z5297	PI 319768	2n=4x=40	AABB (Seijo *et al.* 2004)
A. ipaënsis	30076	PI 468322	2n=2x=20	BB (Robledo and Seijo 2010)
A. batizocoi	9484	PI 298639	2n=2x=20	KK (Robledo and Seijo 2010)
A. hoehnei	30006	PI 468150	2n=2x=20	BB (Krapovickas and Gregory 1994; Holbrook and Stalker 2003)
A. stenophylla	30136	PI 468178	2n=2x=20	EE (Krapovickas and Gregory 1994; Holbrook and Stalker 2003)