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RESEARCH ARTICLE

Rapid gene expression change in a novel synthesized allopolyploid population of cultivated peanut×*Arachis doigoi* cross by cDNA-SCoT and HFO-TAG technique

HE Liang-qiong[1], TANG Rong-hua[1], JIANG Jing[1], XIONG Fa-qian[1], HUANG Zhi-peng[1], WU Hai-ning[1], GAO Zhong-kui[1], ZHONG Rui-chun[1], HE Xin-hua[2], HAN Zhu-qiang[1]

[1] *Cash Crops Research Institute/Guangxi Crop Genetic Improvement and Biotechnology Laboratory, Guangxi Academy of Agricultural Sciences, Nanning 530007, P.R.China*

[2] *Guangxi University, Nanning 530004, P.R.China*

Abstract

Allopolyploidy has played an important role in plant evolution and heterosis. Recent studies indicate that the process of wide hybridization and (or) polyploidization may induce rapid and extensive genetic and epigenetic changes in some plant species. To better understand the allopolyploidy evolutionism and the genetic mechanism of *Arachis* interspecific hybridization, this study was conducted to monitor the gene expression variation by cDNA start codon targeted polymorphism (cDNA-SCoT) and cDNA high-frequency oligonucleotide-targeting active gene (cDNA-HFO-TAG) techniques, from the hybrids (F_1) and newly synthesized allopolyploid generations (S_0–S_3) between tetraploid cultivated peanut Zhongkaihua 4 with diploid wild one *Arachis doigoi*. Rapid and considerable gene expression variations began as early as in the F_1 hybrid or immediately after chromosome doubling. Three types of gene expression changes were observed, including complete silence (gene from progenitors was not expressed in all progenies), incomplete silence (gene expressed only in some progenies) and new genes activation. Those silent genes mainly involved in RNA transcription, metabolism, disease resistance, signal transduction and unknown functions. The activated genes with known function were almost retroelements by cDNA-SCoT technique and all metabolisms by cDNA-HFO-TAG. These findings indicated that interspecific hybridization and ploidy change affected gene expression *via* genetic and epigenetic alterations immediately upon allopolyploid formation, and some obtained transcripts derived fragments (TDFs) probably could be used in the research of molecular mechanism of *Arachis* allopolyploidization which contribute to thwe genetic diploidization of newly formed allopolyploids. Our research is valuable for understanding of peanut evolution and improving the utilization of putative and beneficial genes from the wild peanut.

Keywords: peanut, allopolyploidy, gene expression, start codon-targeted polymorphism, high-frequency oligonucleotide-targeting active gene

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HE Liang-qiong, E-mail: heliangqiong@163.com; Correspondence HE Xin-hua, Mobile: +86-15177169189, E-mail: honest66222@163.com; HAN Zhu-qiang, Mobile: +86-13977139289, E-mail: hanzhuqiang@163.com

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1. Introduction

Polyploidy is a common phenomenon in biological evolution. Most eukaryotes and angiosperms have undergone one or more polyploidization events during their history (Soltis 2005;

Chen 2007; Hollister 2014). Polyploids can be classified into allopolyploids and autopolyploids based on the origins and levels of ploidy. An allopolyploid through interspecific hybridization followed by chromosome doubling has both heterosis and polyploid benefit, thus having been studied in the context of genetics, evolution, molecular biology and breeding (Otto 2007; Wood *et al*. 2009). Two different genomes in the same nucleus often cause genome instabilities, chromosome imbalances and regulatory incompatibilities. Therefore, new synthesized allopolyploids must experience rapid changes and adjustments in genome structure and gene expression in order to establish a compatible relationship (Buggs *et al*. 2012b; Lashermes *et al*. 2014). In recent years, the molecular mechanisms of allopolyploidy evolution have been investigated in some plants. Some polyploid genomes appear to experience rapid genome sequences changes (Gaeta *et al*. 2007; Soltis *et al*. 2010; Kenan-Eichler *et al*. 2011; Buggs *et al*. 2012a; Higgins *et al*. 2012), whereas in other polyploids, gene expression changes appear to dominate (Lee and Chen 2001; Flagel and Wendel 2010; Miller *et al*. 2012), including gene silence, activation and unequal expression of homoeologous gene. However, research on the synthesized allopolyploids cotton and *Gossypium* did not exhibit rapid genomic shock (Liu *et al*. 2001; Baumel *et al*. 2002). Thus, the impact of polyploidization on various plant species is very different. To better understand this phenomenon, further study is needed to conduct using more different plant species allopolyploids.

The *Arachis* section of *Arachis genus* includes nearly 80 diploid and 3 tetraploid species (Talluny 2005). Cultivated peanut (*Arachis hypogaea* L.) is an allotetraploidy through natural hybridization and chromosome doubling, which is grown globally in many countries of Asia and Africa. It is one of the most important oil and economic crop in the world. Most wild species are diploid with resistance to several important pests and pathogens, and usually used for the genetic improvement of cultivated peanut. It is a significant way through interspecific hybridization to utilize wild germplasm. More research on the diploid progenitors of tetraploid cultivar (Gimenes *et al*. 2002; Seijo *et al*. 2004; Bertioli *et al*. 2016) is available, but little is about peanut allopolypoidy evolution mechanism. Gaicia *et al*. (2006) and He *et al*. (2013a, b) reported the relevant research on genome changes in allopolyploidization. Thus, it is necessary to make more related research for understanding the allotetraploid cultivated peanut's evolution mechanism. Synthetic allopolyploids generated from peanut interspecific hybridization permit precise comparison between parents with allopolyploid phenotypes because of clear genetic relationship, which help to provide insights into what kinds of changes in genome sequence and gene expression occurring during allopolyploid evolution. It is valuable to comprehend peanut evolution theory.

Start codon targeted polymorphism (SCoT) and high-frequency oligonucleotides-targeting active gene (HFO-TAG) polymorphisms, both are new functional molecular marker techniques and performed through PCR. SCoT is a simple and novel gene-targeted DNA marker technique developed by Collard and Mackill (2009), which uses a single primer targeting the short conserved region flanking the ATG translation start codon of plant genes, and it has been utilized in genetic diversity studies of rice (Collard *et al*. 2009) and peanut (Xiong *et al*. 2011). HFO-TAG maker technique was developed by Amnon and Willian (2010), and has been used to perform the genotyping of watermelon and its closely related cultivars (Ammon *et al*. 2013). HFO-TAG mainly exists in expressed sequence tag-unigenes with high frequency (Ammon and William 2010; Ammon *et al*. 2013), and can produce more unique and polymorphic amplicons than RAPD and inter-simple sequence repeats (ISSR). HFO-TAG primers consist of 8-, 9- and 10-bp oligonucleotides with 80 to 100% GC content that can enhance the binding targeting power to active gene loci. However, the utilization of cDNA-SCoT and cDNA-HFO-TAG has been barely reported in peanut.

Based on the previous analysis of genome variations in *Arachis* allopolyploidization (He *et al*. 2013a, b), we focus on gene expression variations in the novel synthesized allopolyploid population in the early stages of cultivated peanut Zhongkaihua 4×*Arachis doigoi* cross by using the cDNA-SCoT and cDNA-HFO-TAG, and also verify the feasibility of these two markers. The results are valuable for understanding the mechanism of *Arachis* allopolyploidy evolution and offer a new evidence for the evolution theory of allopolyploid species, which is significant to peanut improvement and wild germplasm utilization.

2. Materials and methods

2.1. Plant materials

Arachis tetraploid cultivar Zhongkaihua 4 (as female) and wild diploid *A. doigoi* (PI 276235, as male) were hybridized in May 2007. The F_1 hybrid was obtained with long growth and flowering period, spreading rapidly by creeping rhizom, amount of branches and flowers without any fruit. The tender branches of F_1 plant were treated with colchicine and cutting propagation. Then the artificial allopolyploid S_0 plants were obtained with restoring fertility. S_1, S_2 and S_3 generations were obtained by S_0 selfing for 1, 2, 3 consecutive generations separately.

F_1–S_2 plants could germinate again in spring of the second year in Nanning of Guangxi, China, where it is not cold in winter. The majority of F_1–S_2 plants were used for