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RESEARCH ARTICLE

Development of SNP markers using RNA-seq technology and tetra-primer ARMS-PCR in sweetpotato

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Abstract

The information of single nucleotide polymorphisms (SNPs) is quite unknown in sweetpotato. In this study, two sweetpotato varieties (Xushu 18 and Xu 781) were sequenced by Illumina technology, as well as *de novo* transcriptome assembly, functional annotation, and *in silico* discovery of potential SNP molecular markers. Tetra-primer Amplification Refractory Mutation System PCR (ARMS-PCR) is a simple and sufficient method for detecting different alleles in SNP locus. Total 153 sets of ARMS-PCR primers were designed to validate the putative SNPs from sequences. PCR products from 103 sets of primers were different between Xu 781 and Xushu 18 *via* agarose gel electrophoresis, and the detection rate was 67.32%. We obtained the expected results from 32 sets of primers between the two genotypes. Furthermore, we ascertained the optimal annealing temperature of 32 sets of primers. These SNPs might be used in genotyping, QTL mapping, or marker-assisted trait selection further in sweetpotato. To our knowledge, this work was the first study to develop SNP markers in sweetpotato by using tetra-primer ARMS-PCR technique. This method was a simple, rapid, and useful technique to develop SNP markers, and will provide a potential and preliminary application in discriminating cultivars in sweetpotato.

Keywords: sweetpotato, SNPs, RNA-seq, tetra-primer ARMS-PCR

1. Introdution

Sweetpotato (*Ipomoea batatas* (L.) Lam.), a member of the family Convolvulaceae, is a dicotyledonous and perennial plant (Austin 1987). This crop is distributed in many countries worldwide, particularly in developing countries, because of its stable yield and wide adaptability. Sweetpotato is also the third most crucial tuber and root crop species after potato and cassava in terms of production. Moreover, for its containing plentiful carbohydrates, vitamins A and C, dietary fiber, iron, potassium, and protein (Bovell-Benjamin 2007), sweetpotato is an important crop for staple food and feed, as well as a major raw material for industries.

Though as one of very important food crops, sweetpotato is lagging in genetic research behind model plants, such as rice (*Oriza sativa* L.), and *Arabidopsis thaliana*. Sweetpotato is a self-incompatible hexaploid plant with high degree of heterozygosity (2n=6X=90)(Ozias-Akins and Jarret 1994), and most economical traits are quantitative (Jones 1986).

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So it is difficult for sweetpotato genetic improvement through conventional techniques. Many kinds of molecular markers have been used for sweetpotato improvement programs and genetic studies in the past few decades (Ukoskit and Thompson 1997; Huang and Sun 2000; Cervantes-Flores *et al.* 2008; Li *et al.* 2008a, b; Schafleitner *et al.* 2010; Wang *et al.* 2010; Moulin *et al.* 2012; Zhao 2012; Zhao *et al.* 2013).

To date, several sweetpotato genetic maps have been developed, but they do not contain sufficient markers to study the genome organization. Therefore, new molecular markers should be discovered. Single nucleotide polymorphisms (SNPs) are among the most common forms of genetic variations, which present a simple change of base pair in the genome. SNPs are abundant in plant genomes, and their biallelic nature allows for a shorter duration of a large-scale genotyping than that of earlier-generation molecular markers. In recent years, SNP-based markers have been widely applied in genomic studies of many plant and animal species. SNP genotyping methods include single-strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis, cleaved amplified polymorphic sequence (CAPS), allele-specific PCR (AS-PCR), SNP arrays, denaturing high-performance liquid chromatography (DHPLC), and high-resolution melting (HRM)(Xu *et al.* 2015b). The next-generation sequencing (NGS) technology is a newly developed method for genome analysis, which can deliver a substantial amount of inexpensive and accurate genome sequence information, and also more effective in producing SNP markers for model and non-model organisms than any existing genetic DNA fingerprinting methods. The RNA-seq method has been used in many crops and can efficiently discover numerous SNP markers (Wu *et al.* 2010; Wei *et al.* 2014; Zhou *et al.* 2014; Chopra *et al.* 2015).

In the previous research, our laboratory used two sweetpotato varieties (Xu 781 and Xushu 18) with distinctly different characters as research objects to describe the *de novo* assembly and annotation of Illumina sequencing reads, and we obtained 1 386 SNPs from the RNA-seq dataset (detailed data not published). We utilized the Tetra-Primer Amplification Refractory Mutation System PCR (ARMS-PCR) procedure (Ye *et al.* 2001) in this study based on our previous experimental results (Xu *et al.* 2015a). Meanwhile, we detected the candidate SNPs between Xu 781 and Xushu 18, and determined the optimal annealing temperature of designed primers from transcriptome data. We also developed some practical and available SNP markers in sweetpotato.

2. Materials and methods

2.1. Plant materials and DNA preparation

Two virus-free sweetpotato varieties, Xushu 18 and Xu 781, were planted in the experimental field of the Sweetpotato Research Institute (Xuzhou of Jiangsu province), Chinese Academy of Agricultural Sciences. Xushu 18 offers high yield, medium dry matter content, good adaptability, high resistance to root rot, and susceptibility to stem nematode. It is also one of the mainly cultivated varieties throughout China. Xu 781, which was selected from the seedlings of the International Potato Center, provides high dry matter content, high resistance to stem nematode, easy flowering, and valuable parental material.

Thirty days after planting, young leaves were selected and frozen immediately in liquid nitrogen. They were ground into powder and stored at −80°C for further use. Total DNA was extracted using a Qiagen DNeasy Plant Mini Kit (Qiagen, Germany) and treated with RNase I (Fermentas, USA) in accordance with the manufacturer's instructions. DNA quality and quantity were determined with 260 nm/280 nm (OD_{260}/OD_{280}) by a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). DNA integrity was assessed by 2.0% agarose gel electrophoresis.

2.2. Design of PCR primers for tetra-primer ARMS-PCR

We previously identified a number of SNP molecular markers between Xushu 18 and Xu 781 based on the SNP information obtained from the sweetpotato transcriptome, and compared different detection methods of SNPs (Xu *et al.* 2015a). The tetra-primer ARMS-PCR technology was selected to assess SNPs because it is fast, simple, and inexpensive, and it can distinguish homozygote from heterozygote SNPs (Ye *et al.* 2001). On the basis of the sequence results of SNP loci, SNP primers were designed by an online program of tetra-primer ARMS-PCR primer design (http://primer1.soton.ac.uk/primer1.html)(Collins and Ke 2012). Total 153 sequences containing SNPs were randomly chosen, and the corresponding tetra-primer ARMA-PCR primer sets were validated to investigate the genetic differences between the two selected sweetpotato varieties (Xushu 18 and Xu 781). The fragment sizes of PCR products were maintained within the range of 150–500 bp. We designed two outer primers (FO and RO) and two allele-specific inner primers (FI and RI) for each selected SNP locus. Simultaneously, a mismatch base was introduced at the 3′ end of each of the two allele-specific primers to increase the specificity of the reaction (Hayashi *et al.* 2004). Primers were synthesized by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd. (China).

2.3. Tetra-primer ARMS-PCR amplification

Tetra-primer ARMS-PCR amplification was performed using the primer sets designed as described above. PCR amplification was conducted in a 15-μL reaction containing 50 ng of template DNA, 1 μL of each inner primer (10 μmol L^{-1}),

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