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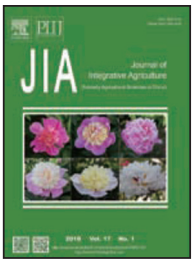

RESEARCH ARTICLE

SSR fingerprinting of 203 sweetpotato (*Ipomoea batatas* (L.) Lam.) varieties

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Abstract

Simple sequence repeat (SSR) markers have been shown to be a powerful tool for varieties identification in plants. However, SSR fingerprinting of sweetpotato varieties has been a little reported. In this study, a total of 1 294 SSR primer pairs, including 1 215 genomic-SSR and 79 expressed sequence tag (EST)-SSR primer pairs, were screened with sweetpotato varieties Zhengshu 20 and Luoxushu 8 and their 2 F_1 individuals randomly sampled, and 273 and 38 of them generated polymorphic bands, respectively. Four genomic-SSR and 3 EST-SSR primer pairs, which showed good polymorphism, were selected to amplify 203 sweetpotato varieties and gave a total of 172 bands, 85 (49.42%) of which were polymorphic. All of the 203 sweetpotato varieties showed unique fingerprint patterns, indicating the utility of SSR markers in variety identification of this crop. Polymorphism information content (PIC) ranged from 0.5824 to 0.9322 with an average of 0.8176. SSR-based genetic distances varied from 0.0118 to 0.6353 with an average of 0.3100 among these varieties. Thus, these sweetpotato varieties exhibited high levels of genetic similarity and had distinct fingerprint profiles. The SSR fingerprints of the 203 sweetpotato varieties have been successfully constructed. The highly polymorphic SSR primer pairs developed in this study have the potential to be used as core primer pairs for variety identification, genetic diversity assessment and linkage map construction in sweetpotato and other plants.

Keywords: EST-SSR, fingerprinting, genetic distance, genomic-SSR, sweetpotato

1. Introduction

Sweetpotato, *Ipomoea batatas* (L.) Lam., is the seventh most important food crop worldwide and its annual production reaches 104.46 million tons globally (FAO 2014). In many developing countries, sweetpotato is a staple food crop because it produces large quantities of energy per day (Hagenimana and Low 2000; Srinivas *et al*. 2009; Li *et al*. 2014). Sweetpotato is a vegetatively propagated species, and each variety is a clone. Phenotypic markers are usually used to provide descriptors for identifying sweetpotato varieties, but they are unreliable due to their paucity and vulnerability to environmental influence (Prakash *et al*. 1996).

Molecular marker techniques have been proved to be powerful tools for identifying plant species, varieties, clones, individuals and even plant products (Karihaloo 2015). Prakash *et al*. (1996) used DNA amplification fingerprinting (DAF) to investigate genetic relationships of sweetpotato

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varieties and developed fingerprint profiles of the 30 USA varieties with 7 octamer primers. Guo *et al*. (2003) and Wang H Y *et al*. (2009) constructed random amplified polymorphic DNA (RAPD) fingerprints of the 10 and 30 sweetpotato varieties with one RAPD primer, respectively. In the study of Liu *et al*. (2012), the 98 sweetpotato varieties were distinguished with one amplified fragment length polymorphism (AFLP) primer combination. Simple sequence repeat (SSR) is short tandem nucleotide repeats and is known for its high polymorphism level (Hayden and Sharp 2001; Chen H L *et al*. 2016; Dos Santos *et al*. 2016). SSR markers are easy to detect, highly informative, stable, and comparatively inexpensive to use in research, and have been shown to be a powerful tool for varieties identification in several plant species such as potato (Moisan-Thiery *et al*. 2005), cabbage (Louarn *et al*. 2007), tomato (Caramante *et al*. 2011), rose (Akond *et al*. 2012), mango (Kumar *et al*. 2013), rice (Jiang *et al*. 2010; Lu *et al*. 2014) and oil camellia (Chen Y N *et al*. 2016). To date, SSR markers have been used to assess the genetic diversity of accessions and construct genetic linkage maps in sweetpotato (Hwang *et al*. 2002; Zhao *et al*. 2013; Hundayehu *et al*. 2014; Koussao *et al*. 2014; Rodriguez-Bonilla *et al*. 2014; Yada *et al*. 2015; Yang *et al*. 2015; Ngailo *et al*. 2016). However, the SSR fingerprinting of sweetpotato varieties has been a little reported. Luo *et al*. (2014) constructed the DNA fingerprinting of 52 sweetpotato accessions with 2 expressed sequence tag (EST)-SSR primers.

In this study, 4 genomic-SSR and 3 EST-SSR primer pairs were selected from 1 215 genomic-SSR and 79 EST-SSR primer pairs, respectively, to construct the fingerprints of the 203 sweetpotato varieties. All of the varieties showed unique fingerprint patterns, which indicates the utility of SSR markers in variety identification of sweetpotato.

2. Materials and methods

2.1. Plant materials

Sweetpotato varieties Zhengshu 20 and Luoxushu 8 and their 2 F_1 individuals randomly sampled, which were provided by the Crop Research Institute, Shandong Academy of Agricultural Sciences, China, were used for screening polymorphic SSR primer pairs. The 2 varieties have distinct differences in yield, starch content and diseases resistance. For SSR fingerprinting, the 203 sweetpotato varieties were collected from China and Japan (Appendix A).

2.2. DNA extraction

Genomic DNA from each variety was extracted from young leaves with the cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof *et al*. 1984). The DNA quality was quantitated on a 1% (w/v) agarose gel, and the DNA concentration of each sample was quantified under UV illumination.

2.3. SSR primer designing and screening

A total of 1 215 genomic SSR primer pairs with di-, tri- and tetra-nucleotide motifs were developed from whole genome sequencing of sweetpotato *cv*. Xushu 18. These genomic SSR primer pairs were provided by Dr. Sachiko Isobe, the Kazusa DNA Research Institute, Japan. Meanwhile, a total of 6 130 EST sequences were downloaded from the EST database of NCBI (http://www.ncbi.nlm.nih.gov/nucest). After removing redundant sequences, the remaining sequences were used to search EST-SSR sequences by software AutoSSR (Wang C B *et al*. 2009), and the number of repeats containing di-, tri-, tetra-, penta-, and hexa-nucleotide motifs was 9, 6, 5, 4 and 3, respectively. The selected EST-SSR sequences longer than 150 bp in general were used to design SSR primers by Primer Premier 5.0 according to the following standard parameters: target amplicon lengths of 100–900 bp, annealing temperatures of 55–65°C, GC contents of 40–60% and primer sizes of 18–28 bp. A total of 79 EST-SSR primer pairs were designed. All of the genomic-SSR and EST-SSR primer pairs were synthesized at BGI-Tech (Shenzhen, China).

A total of 1 294 SSR primer pairs were screened with Zhengshu 20 and Luoxushu 8 and their 2 F_1 individuals randomly sampled to detect their polymorphism. PCR amplification was performed in a 20-μL reaction solution consisting of 3 μL (50 ng μL^{-1}) DNA template, 2 μL 10× PCR buffer, 0.8 μL (10 mmol L^{-1}) dNTPs, 0.2 μL (5 U μL^{-1}) Easy*Taq*® DNA polymerase (TransGen Biotech, Beijing, China), 1 μL (10 μmol L^{-1}) of each SSR primer (BGI-Tech, Shenzhen, China) and 12 μL deionized distilled water. PCR conditions for amplification were as follows: 95°C for 5 min followed by 35 cycles at 95°C for 1 min, 56.8–59°C (depending on the SSR primers, Table 1) for 30 s, 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products were denatured and analyzed by Bio-Rad (USA) electrophoresis on 6% denaturing polyacrylamide gel in 1× TBE buffer and visualized by silver staining.

2.4. SSR fingerprinting

Genomic-SSR and EST-SSR primer pairs showing good polymorphism were selected to amplify the 203 sweetpotato varieties for constructing their fingerprint profiles as described above. Polymorphic bands were visually scored as binary data with presence as “1” and absence as “0” by GeneRuler™ 100 bp DNA Ladder.

Characteristics of the SSR primer pairs for constructing