IDENTIFICATION OF ISSR PRIMERS FOR GENETIC ANALYSIS OF *TELFAIRIA OCCIDENTALIS* HOOK F.

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(Received: August, 2010; Accepted: May, 2011)

ABSTRACT

Telfairia occidentalis Hook f. (commonly called fluted pumpkin) is found in the moist coastal areas of West Africa, especially in Nigeria. It is a widely-eaten leaf and seed vegetable which belongs to the family Cucurbitaceae. A study was conducted to screen ISSR primers to select those that can amplify DNA extracted from ***Telfairia occidentalis***. Eight ISSR primers were screened at different temperatures in order to identify primer and optimal temperature that will produce clear and reproducible bands on 2% agarose gels. Two of the ISSR primers (ISSR2 & ISSR4) produced bands at annealing temperatures varying from 56^0C to 66^0C and 50^0C to 55^0C respectively. The optimal annealing temperature (Ta) 60^0C was identified for ISSR2 and 53.1^0C was identified for ISSR4.

ISSR2 and ISSR4 are considered promising for the study of genetic diversity in ***Telfairia occidentalis***.

Keywords: Vegetable, *Telfairia occidentalis*, ISSR Primers, DNA

INTRODUCTION

Telfairia occidentalis commonly called fluted pumpkin belongs to the family Cucurbitaceae. The highly nutritious leaves and seeds make it one of the three most important and widely eaten vegetables in West Africa (Abiose, 1999). Among the different genotypes that exist, it is difficult to identify those that condition the broad succulent leaf preferred by consumers. These genotypes could be valuable sources of genetic diversity that can be used in the development of improved cultivars for commercialization (Odiaka, 2005).

Molecular methods are modern tools which assist in a better understanding of genetic diversity. Bornet *et al.* (2002) reported Inter Simple Sequence Repeat (ISSR) sequences as molecular markers that can lead to the detection of polymorphism and also a new approach to study SSR distribution and frequency. ISSR, which is a dominant marker like RAPD, has greater robustness in repeatability and high variability it could therefore be used to study genetic diversity among African edible cucurbits (Dje *et al.*, 2006). However, since there is a lot of diversity among plants, primers that work for one may not work for another. Hence, ISSR primers need to be screened and optimized for specific species. The objective of this study was to screen ISSR primers in order to select those that can amplify DNA extracted from ***Telfairia occidentalis***.

MATERIALS AND METHODS

Plant material

Telfairia occidentalis seeds collected in Ile-Ife, Nigeria were used in this study. The seeds were grown in a greenhouse at the School of Biological & Conservation Sciences, University of Kwazulu-Natal, Durban, South Africa. Ten individual plants were sampled.

DNA extraction

DNA was extracted using the DNeasy plant mini/maxi kit. About 55 to 80 mg of young leaves from 14 day-old plants were macerated in liquid nitrogen to fine powder and thereafter transferred to Eppendorf tubes without thawing. The DNA was then extracted using the DNeasy plant mini/maxi kit according to the manufacturer's instruction. The extracted DNA was quantified using a Nanodrop ND 1000 spectrophotometer.

PCR and electrophoresis

A total of eight ISSR primers were screened in order to select those that could amplify the extracted DNA. The PCR amplification was aimed at the selection of ISSR primers giving few discrete, large bands using several annealing temperatures. A total volume of 25 μL of a solution containing 9 μL of sterile water, 2.4 μL of PCR buffer (from PCR kit), 3 μL of $MgCl_2$ (25mM), 1.2 μL of each ISSR primer, 0.2 μL of 100 mM dNTPs, 0.2 μL of Taq DNA polymerase (from PCR kit) and 9 μL PCR grade water

containing 20ng extracted DNA was prepared. PCR cycling conditions were at 94°C for 3mins, 33 cycles of (denaturing at 94°C for 2mins, 2mins at the annealing temperature, and elongation at 72°C for 3 mins) and final elongation at 72°C for 7 mins). All PCR reactions were performed with thermocycler MJ mini PTC (BioRad). The result of each amplification reaction was analyzed on 2% agarose gel in 1X TBE buffer and run at 80 V for 5 hours. Thereafter, amplified fragments were visualized using an ultraviolet trans-illuminator and on Video Copy Processor.

RESULTS AND DISCUSSION

Using the primers to amplify ISSR sequences in DNA extracted from *Telfairia occidentalis*, two of the eight ISSR primers from the PCR produced positive results from the PCR trials (Table 1). The two primers (ISSR4 and ISSR2) produced bands at annealing temperatures 53.1^{0}C and 60^{0}C respectively (Table 2).

Biological diversity is a commonly recognized value in natural resources management and recognizing and managing the genetic diversity within a species is valuable (Rogers, 2006). According to Kresovich *et al.* (1995), technologies based on molecular markers provide the only tools that are able to reveal polymorphism at the DNA sequence level and these can detect genetic variability between individuals and within populations.

The female plants of *Telfairia occidentalis* are the ones that are economically important and it will be beneficial to have a molecular marker that can be correlated with morphological markers to distinguish between male and female plants at the seedling stage.

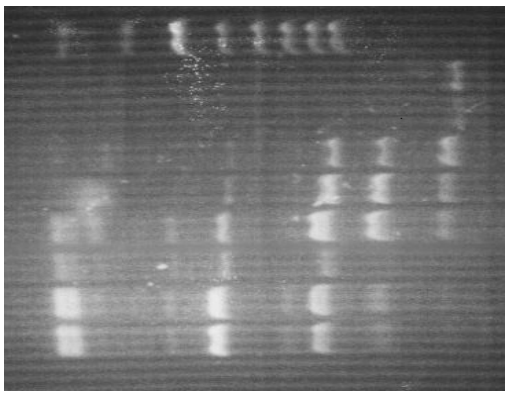
From this study, 2 primers that can be used in the study of *T. occidentalis* have been identified. However, other primers also need to be screened in order to identify those suitable for the PCR amplification of *T. Occidentalis*.

Table 1: List of ISSR Primers Used

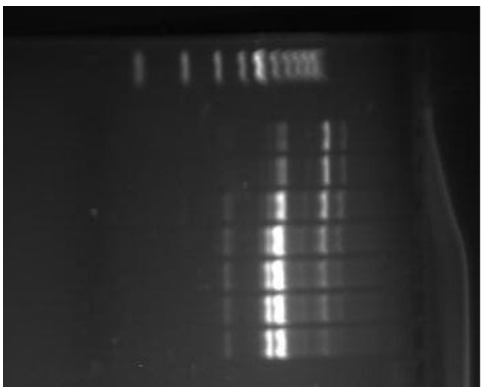
S/N	PRIMERS	SEQUENCES	TEMPERATURE RANGE(°C)
1	ISSR1	$(CAA)_5$	50 – 55
2	ISSR2	$(CAG)_5$	56 - 66
3	ISSR3	$(GATA)_4$	41 -56
4	ISSR4	$(GACA)_4$	50 -55
5	ISSR5	$(CCTA)_4$	36 - 46
6	ISSR6	$(GA)_9T$	56 - 66
7	ISSR7	$(GCAT)_4$	41 - 56
8	ISSR8	$(TAG)_4$	36 - 46

Table 2: List of Selected Primers and Optimal Annealing Temperatures Obtained Using *Telfairia*

S/N	PRIMERS	SEQUENCES	Ta
1	ISSR2	$(CAG)_5$	60^{0}C
2	ISSR4	$(GACA)_4$	53.1^{0}C



ISSR 2



ISSR 4

ACKNOWLEDGEMENTS

I will like to acknowledge the assistance of Dr Kunle Adebowale, Prof Norman Pammenter, Prof Pat Berjak, Prof. J. Lamb, Jessica, Prisha Naidoo, Madhupa and Dr Bayo Shittu for access to laboratory facilities, training in different techniques and editing of manuscript. This work was made possible by a fellowship for Female Academic Staff from Carnegie Corporation, USA, through the Centre for Gender & Social Policy Studies, Obafemi Awolowo University, Ile-Ife, Nigeria.

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Ife Journal of Science

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