INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

THE DYNAMICS AND COMPARISON OF TRANSPOSABLE ELEMENTS FROM CRYPTIC PLANT SPECIES

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Article Received on: 15/12/12 Revised on: 01/01/13 Approved for publication: 10/02/13

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ABSTRACT

The purpose of the study was to evaluate the possible genetic variation occurred due to the action of transposons. *Hibiscus rosasinensis* was selected for the study as it had the transposable elements similar to the one found in maize, which can be useful for the differentiation of colors in this plant. In this study an attempt was made to identify the transposable elements and to study the dynamics of the cryptic plant species. The plant DNA was successfully extracted and run with 0.8% Agarose gel electrophoresis. After isolation of DNA specific transposable element primers were used to amplify the 300bp of fragment. These fragments were subjected to RAPD which identified the transposon sequence (106bp) named as *En/Spm*. These fragments represent ribulose 1, 5-bisphosphate carboxylase large subunit (rbcL) gene which were confirmed with NCBI-BLAST. The sequences were initially aligned with Clustal-X software. Based on this alignment, 224bp of sequence were used to construct phylogenetic tree.

Hence, the relationship shows that *Hibiscus rosasinasesis* (Brown) and *Hibiscus rosasinensis* (Pink) are closely related, but the *Hibiscus* rosasinasesis (Red) is distantly related. The same results were shown in RAPD technique when the transposable element sequence was compared with RAPD marker. It can be concluded that the *Hibiscus rosasinensis* plant has more than 80% of transposons which was responsible for the color changes.

Keywords: transposable elements, *Hibiscus rosasinensis*, ribulose 1, 5-bisphosphate carboxylase large subunit gene, genetic application.

INTRODUCTION

Hibiscus rosasinensis L. (Malvaceae) is widely appreciated as ornamental shrubs in tropical and sub-tropical regions[1]. *Hibiscus rosasinensis* is one of the plant genera with a genetic characteristic known as polyploidy in which two complete set of chromosomes differ from each other. Since, *Hibiscus rosasinensis* has the polyploidy characteristic; it became popular variety in holding strikingly unique flowers.

The molecular approach for studying a plant species is to understand the relationship between plants. RAPD (Random Amplified Polymorphic DNA) technique has been widely used in the analysis of Genetic linkage mapping, although the RAPD method uses arbitrary primer sequences, many of these primers must be screened in order to select primers that provides useful amplification products. In contrast, the molecular markers facilitate research on genetic variation at the DNA level.

In the present research, we analyze the optimization of primer screening and evaluation of genetic variation among the three species of *Hibiscus rosasinensis* L. by using RAPD, Clustal-X and Phylogenetic tree.

MATERIALS AND METHODS

Collection of Plant Species

Young leaves of *Hibiscus rosasinensis* red, *Hibiscus rosasinensis* pink and *Hibiscus rosasinensis* brown plants were collected.

Chemicals Required

- C-TAB (hexadecyl Trimethyl Ammonium Bromide)
- Extraction buffer
- Sodium acetate
- Ice cold alcohol
- TE BUFFER
- TAE BUFFER
- Chloroform and alcohol

DNA Extraction: (CTAB method).

Weighed 150mg of freshly collected leaves and homogenized well. Added 500µl of extraction buffer made from CTAB [2]. These tubes were kept in water bath at 60^0c for 1hour. Centrifuged all the tubes at 10,000 rpm for 5 minutes at 4˚C.Transfered the supernatant into the fresh vial and added 480µl of chloroform and 20µl 0f Iso-amyl alcohol . Centrifuged at 12000 rpm for 15 minutes at 4˚C and collected the aqua phase and transferred that into a new vial and added 100µl of 3M Sodium acetate and 500µl of Ice cold ethanol. Centrifuged at 12000 rpm for 10 minutes at 4˚C and Collected the pellet added 800µl of 70% ethanol. Then centrifuged at 10,000 rpm for 5 minutes at 4˚C and discarded the alcohol and air dried the pellet and added 40µl of TE buffer to the microfuge tubes. Then Run the DNA in the Electrophoretic gel.

Agarose Gel Preparation

Weighed 0.8% (0.16g) of Agarose and dissolved in 20.0ml distilled water, heated till the solution becomes clear and added 10.0µl ETBR(Ethidium Bromide) [2&3] and casted in the electrophoresis boat.

DNA Sequencing

The 300bp of amplified DNA fragment was sequenced using the ABI 3130 Genetic Analyzer (Big Dye Terminator version 3.1 Cycle sequencing kit from Bio-serve India Pvt.ltd Hyderabad). Then the sequences were confirmed with NCBI-BLAST followed by aligning with Clustal-X software (Bio-informatics tool) and the phylogenetic tree was constructed with MEGA4.0 software[4, 5, 6,7] .

RESULTS

The plant DNA was successfully extracted and run with 0.8% Agarose gel electrophoresis[2,3]. As indicated in figure 1 the DNA was isolated according to their molecular weight.

After isolation of DNA specific transposable element primer were used to amplify the 300bp of fragment (fig2)[8]. RAPD

technique was used to compare the transposable element sequence and RAPD marker.

The transposable element fragments represent ribulose 1,5-bisphosphate carboxylase large subunit (rbcL) gene which were confirmed with NCBI-BLAST. These sequences were initially aligned with Clustal-X software (fig3) (Bio-informatics tool) which is found to be responsible for the color changes. These sequences were named as *En/Spm.*

Based on the alignment, 224bp of sequence were used to construct phylogenetic tree. The relationship shows that *Hibiscus rosasinasesis* (Brown) and *Hibiscus rosasinensis* (Pink) are closely related, but the *Hibiscus* rosasinasesis (Red) is distantly related.

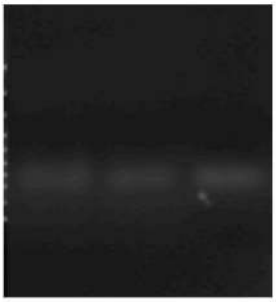


Figure1 Plant DNA bands according to their molecular weight

Content	Abbreviations
HB	*Hibiscus rosasinensis.* BROWN
HP	*Hibiscus rosasinensis* PINK
HR	*Hibiscus rosasinensis* RED
M	MARKER

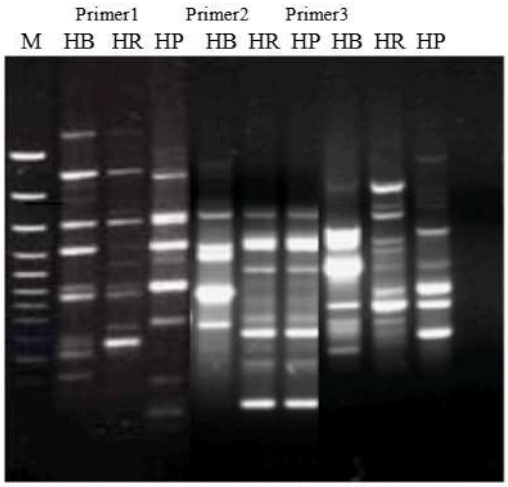


Figure 2 Amplified DNA base pairs according to their primers and hibiscus species

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                    10         20         30         40
                    ....|....|....|....|....|....|....|....|....|
HB sequence.        AAAGCTTTACGTATGTCTGGTGGAGATCATATTCACGCTGGTACA
HP Sequence.        AAAGCTTTACGTATGTCTGGTGGAGACCATATTCACGCTGGTACA
HR Sequence.        AAGGCTTTACGTATGTCTGGTGGAGATCATATTCACGCTGGTACA

                   110        120        130        140
                    ....|....|....|....|....|....|....|....|....|
HB sequence.        TACGTGATGATTTTATTGAAAAAGATTCATTCCGTGGTATTTATT
HP Sequence.        TACGTGATGATTTTATTGAAAAAGATTCATTCCGTGGTATTTATT
HR Sequence.        TACGTGATGATTTTATTGAAAAAGATTCATTCCGTGGTATTTACT

                   210        220
                    ....|....|....|....|....
HB sequence.        TCACGTTTGGCATATGCCTGCTTT
HP Sequence.        TCACGTTTGGCATATGCCTGCTTT
HR Sequence.        TCACGTTTGGCATATGCCTGCTTT
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Figure 3 Hibiscus plant sequences based on Clustal-X software

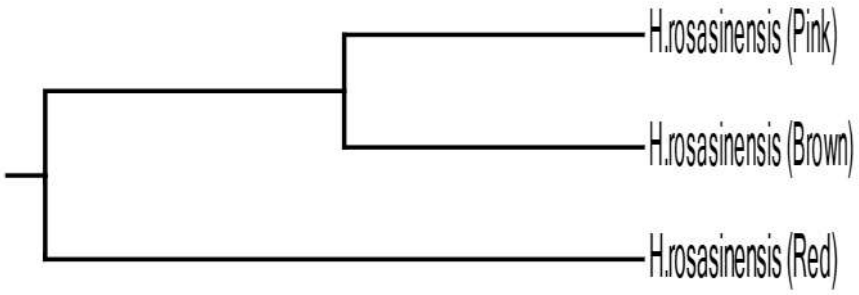


Figure 4 Phylogenetic relation among *Hibiscus rosasinensis*

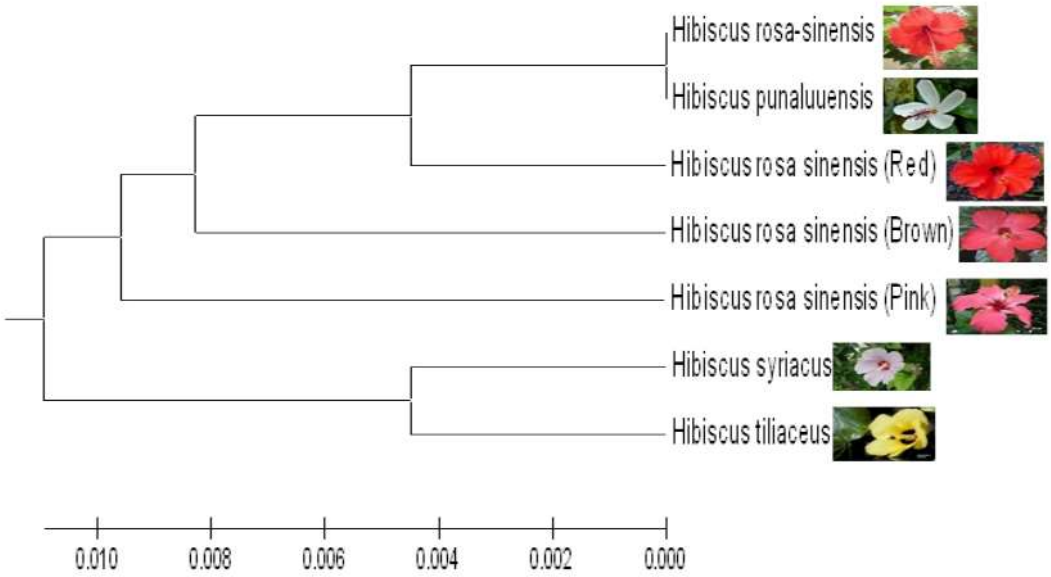


Figure 5. Pictorial representation of phylogenetic relation between Hibiscus species.

DISCUSSION

In this molecular approach, the transposable element is the source which is responsible for the color change in *Hibiscus rosasinensis* plant. Similar case was proved in Evolutionary conservation of angiosperm flower development[9]. Here *En/Spm* is the responsible for the evolutionary changes. Hence, *Hibiscus rosasinensis* red is distantly related when compared with *Hibiscus rosasinensis* Brown and *Hibiscus rosasinensis* Pink which are closely related.

ACKNOWLEDGEMENT

Our special thanks to CBNR (Center for Bioscience & Nano- Science Research Center) for supporting the work.

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Source of support: Nil, Conflict of interest: None Declared

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