iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 5 **Issue:** XI **Month of publication:** November 2017

DOI:

www.ijraset.com

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor:6.887
Volume 5 Issue XI November 2017- Available at www.ijraset.com

Studies on Genetic Diversity of Citrus Species Prevalent in Assam

Ajanita Mazumdar[1], Trishala Das[2]
[1] *Department of Biotechnology, Cotton University, Guwahati, India*

Abstract: ***With high demand and popular dietary preference, citrus fruit is widely consumed and has become an inseparable part of our diet. Characterized by their distinct aroma and delicious taste, it has been recognized as an important food, playing key roles in supplying energy and nutrients. In Assam various species of Citrus have been cultivated locally which sums up to the mass yield to respond to the increasing need to feed the world population. Considering the importance of citrus plants, the present study was undertaken to determine the inter specific diversity amongst the Citrus species in terms of molecular aspects using restriction profiling .Six locally available Citrus species were selected based on the popularity of the fruits which include C.limon (Lemon), C.aurantifolia (Keylime), C.sinensis (Orange), C.maxima (Pomelo), C.limetta (Mosambi), C.medica (Citron). Molecular weights in terms of base pairs were calculated comparing to a standard DNA marker. C.medica was found to have highest molecular weight of ~ 4000 base pairs followed by C.maxima with molecular weight of ~3800 base pairs .C.limon and C.limetta showed DNA bands of similar size of around 3700 base pairs. C.sinensis showed a band size of ~3500 base pairs. C. aurantifolia showed the lowest molecular weight of ~3200 base pairs. Restriction digestion of the samples showed bands of different molecular weights indicating interspecific diversity.***

Keywords: Citrus sp., Genomic DNA, Interspecific diversity, DNA marker, Restriction digestion

I. INTRODUCTION

Fruits are the major contributors of nutrients in our dietary system. With high demand and popular dietary preference, citrus fruit is widely consumed and has become an inseparable part of our diet. Citrus fruits are abundant in vitamin C, simple sugars and dietary fiber, folate, thiamin, niacin, vitamin B_6, riboflavin, pantothenic acid, potassium, calcium, phosphorus, magnesium, and copper [9]. In addition, naturally occurring photochemical, including limonoids, flavonoids and carotenoids are also present. These biological constituents are of vital importance in human health improvement due to their antioxidant properties [9]. Some epidemiological studies report a 40% to 50% reduced risk of certain cancers with increased citrus consumption [9], [10].

There are four original citrus fruits : citron, pomelo, mandarin and papeda from which the most of the other types are developed through natural hybrid speciation or artificial hybridization [10]. Some of such species of Citrus include Citrus aurantiifolia,Citrus crenatifolia ,Citrus mangshanensis ,Citrus medica,Citrus latipes ,Citrus reticulate, Citrus trifoliata, Citrus indica.Hybrid cultivar such as Kinnow ,Sweet orange,Lemon etc.Some of the species which are prevalent in Assam are C.limon(Lemon),C.aurantifolia (Key lime.),C.sinensis(Orange),C.maxima (Pomelo),C.limetta (Mosambi),C.medica (Citron). It was hypothesized that though these fruits belong to the Citrus genera, significant genetic diversity will be present within these fruits as their origin and distribution are diversed. Considering the importance of the citrus fruits and availability in the state of Assam, the present investigation was carried out to study the genetic diversity amongst some selected Citrus species of Assam through the isolation and restriction digestion of their genomic DNA.

II. MATERIALS AND METHODS

A. Sample Collection

Six locally available *Citrus* species was selected based on the popularity of the fruits in the state of Assam. Those are *C.limon* (lemon), *C.aurantifolia* (Key lime.), *C.sinensis* (Orange), *C.maxima* (Pomelo), *C.limetta* (Mosambi) ,*C.medica*(Citron). The leaves of those selected fruits were collected from the local areas of Guwahati, Assam during the month of January in the year 2016.

B. Extraction Of DNA From The Samples

Isolation of DNA was carried out adding 10mM of Lysis buffer(To make 100 ml of lysis buffer, 0.372g of EDTA and 0.5g of SDS was dissolved in 100ml of 10 mM Tris-HCl ,pH 8)to 2g of each of the finely ground samples. Samples transferred to centrifuge

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tubes were incubated for 30 minutes at 65^{0}C. Tubes were centrifuged at 10,000rpm for 6 minutes. Supernatant was pipetted out to which 500µl of chilled chloroform: is amyl alcohol(24:1) was added and mixed gently. Centrifugation was repeated. The aqueous layer was pipetted out and absolute chilled alcohol was added followed by centrifugation. Tubes were then decanted. 70% ethanol was added to each of the tubes and air dried. The DNA pellets were then suspended in TE buffer and stored at 4^{0}C for further use [5], [7].

C. Restriction digestion of the samples

Two restriction enzymes BamH1 and HindIII were used for the digestion of the samples [1], [2], [4].The preparations for digestion of samples with BamH1 and Hind III are described in Table I and Table II respectively. Eppendorf tubes containing the total mixture were incubated overnight at 35^{0}C in a hot water bath**.**

TABLE I: PREPARATION FOR DIGESTION OF SAMPLES WITH BAM H1

Sl.No	Sample (µl)	DNA conc. (µl)	Master mixture(µl)	DNAse free water(µl)	Total mixture(µl)
1	C.medica	10	15	15	40
2	C.aurantifolia	10	15	15	40
3	C.limetta	10	15	15	40
4	C.limon	10	15	15	40
5	C.sinensis	10	15	15	40
6	C.maxima	10	15	15	40

TABLE II:PREPARATION FOR DIGESTION OF SAMPLES WITH BAM H1

S.No	Sample (µl)	DNA conc. (µl)	Master mixture(µl)	DNAse free water(µl)	Total mixture(µl)
1	C.medica	10	15	15	40
2	C.aurantifolia	10	15	15	40
3	C.limetta	10	15	15	40
4	C.limon	10	15	15	40
5	C.sinensis	10	15	15	40
6	C.maxima	10	15	15	40

D. Agarose Gel Electrophoresis Study of the Samples

0.8% agarose gel was prepared.10µl of extracted DNA samples were dissolved in 10µl of 6X gel loading buffer and subjected to agarose gel electrophoresis for 1hour 45 minutes at 100V in order to quantify the pure form of genomic DNA . Similarly, 10µl of each sample digested with restriction enzymes were also dissolved in 10µl of 6X gel loading buffer and gel electrophoresis was carried out for 1 hour 45 minutes at 100V to assess the fragments of DNA thus obtained. The DNA bands were visualized under an UV Tran illuminator.

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III. RESULTS

A. Isolation Of Genomic DNA

DNA was successfully isolated from the leaf samples. The size of the genomic DNA of each sample was compared with that of Hind III digested lambda DNA marker (Fig. 1). It was found that the molecular weight of C.medica was the highest, ~ 4000 bp followed by C.maxima with molecular weight ~3800 bp .C.limon and C.limetta showed similar sized DNA.i.e ~3700bp.C.sinensis showed a band size of ~3500.C.aurantiifolia showed the lowest molecular weight of ~3200.

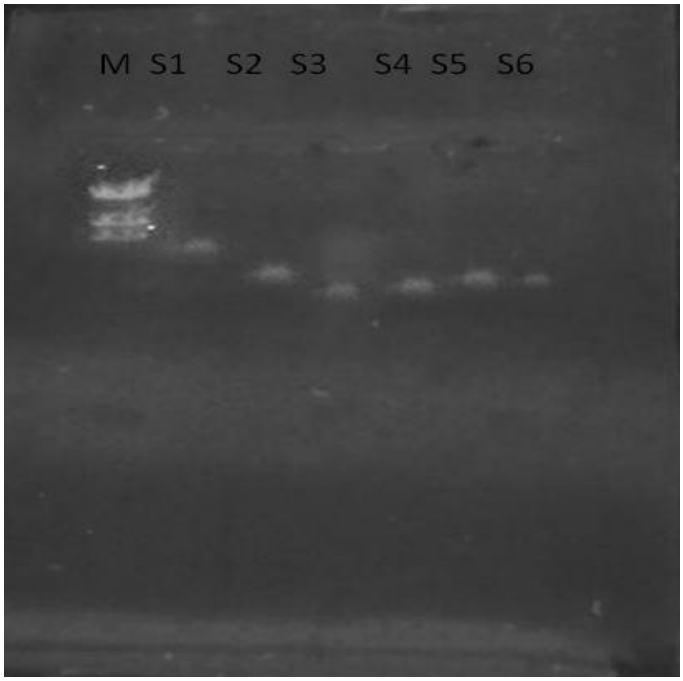


Fig. 1Isolation of genomic DNA from the samples: M: DNA marker, S1: C.medica, S2: C.aurantiifolia, S3:C.maxima, S4:C.limetta, S5:C.limon, S6:C.sinensis.

B. Restriction Digestion Using Restriction Enzymes

Restriction digestion of the samples was carried out using BamHI and HindIII. Bands of different molecular weights were observed with different restriction enzymes in each sample (Fig. 2 and Fig. 3).

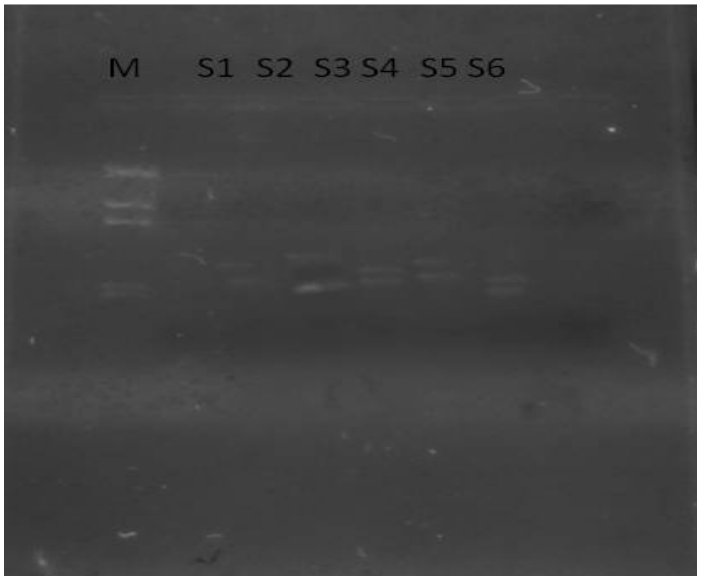


Fig. 2 Restriction digestion of the samples using BamHI. : DNA Marker, S1: C.medica ,S2: C.aurantiifolia ,S3: C.limetta, : C.limon , S5: ,C.sinensis, S6: C.maxima.

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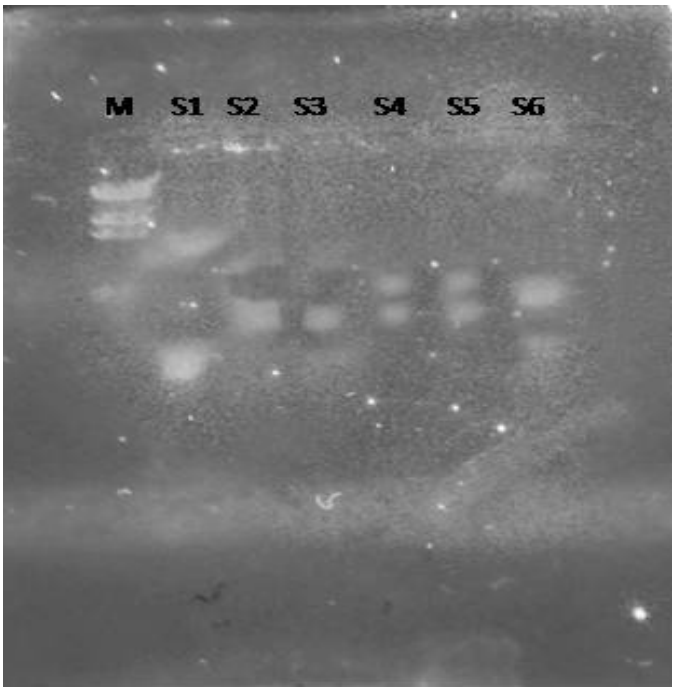


Fig. 3Restriction Digestion of the samples using HINDIII. DNA Marker, S1: C.medica, S2: C.aurantiifolia , S3: C.limetta, : C.limon , S5: ,C.sinensis, S6: C.maxima.

IV. DISCUSSIONS

Citrus fruits are among the most widely grown and economically important fruit tree crops in the world. As fresh fruit, they are an important and nutrient dense food source for human diets thus they represent a globally traded commodity.

The present study was undertaken to determine the variation among six species of the genus Citrus using DNA profiling with a view to appraising genetic diversity and phylogeny within the genus Citrus. The weights of DNA was found to be ~4000 bp in case of C.medica followed by C.maxima with molecular weight ~3800 bp .C.limon and C.limetta showed similar sized DNA .i.e ~3700 bp.C. sinensis showed a band size of ~3500.C.aurantifolia showed the lowest molecular weight of ~3200. It is reported that C.medica , has an average value of 398 Mb/ haploid genome, C.maxima had an average of 383 Mb/haploid genome followed by 370, 368, and 380 Mb for C. sinensis, C. aurantium and C. limon. haploid genomes, respectively [1], [3], [6], [9].Restriction digestion shows different restriction sites in each sample yielding DNA fragments of various molecular weights thus depicting their interspecific diversity though belonging to the same genus Citrus. Genetic diversity was studied successfully using restriction mapping in Rice [8], [11]. But unfortunately no works have been reported on determination of genetic diversity through restriction profiling of the inCitrus sp. Specific variation on Citrus spp. was studied by techniques using RAPD, ISSR,IRAP and REMAP markers [2].

V. CONCLUSION

Citrus fruit makes it stand high among fruit crops. The fruit has been recognized as an important food and integrated as part of our daily diet, playing key roles in supplying energy and nutrients and in health promotion. Although citrus taxonomy has been well studied ,confusion remains regarding the number of true Citrus species and their relationship with each other. Various techniques of RFLP,RAPD, AFLP,SNPs markers are being used form genomic studies of Citrus.

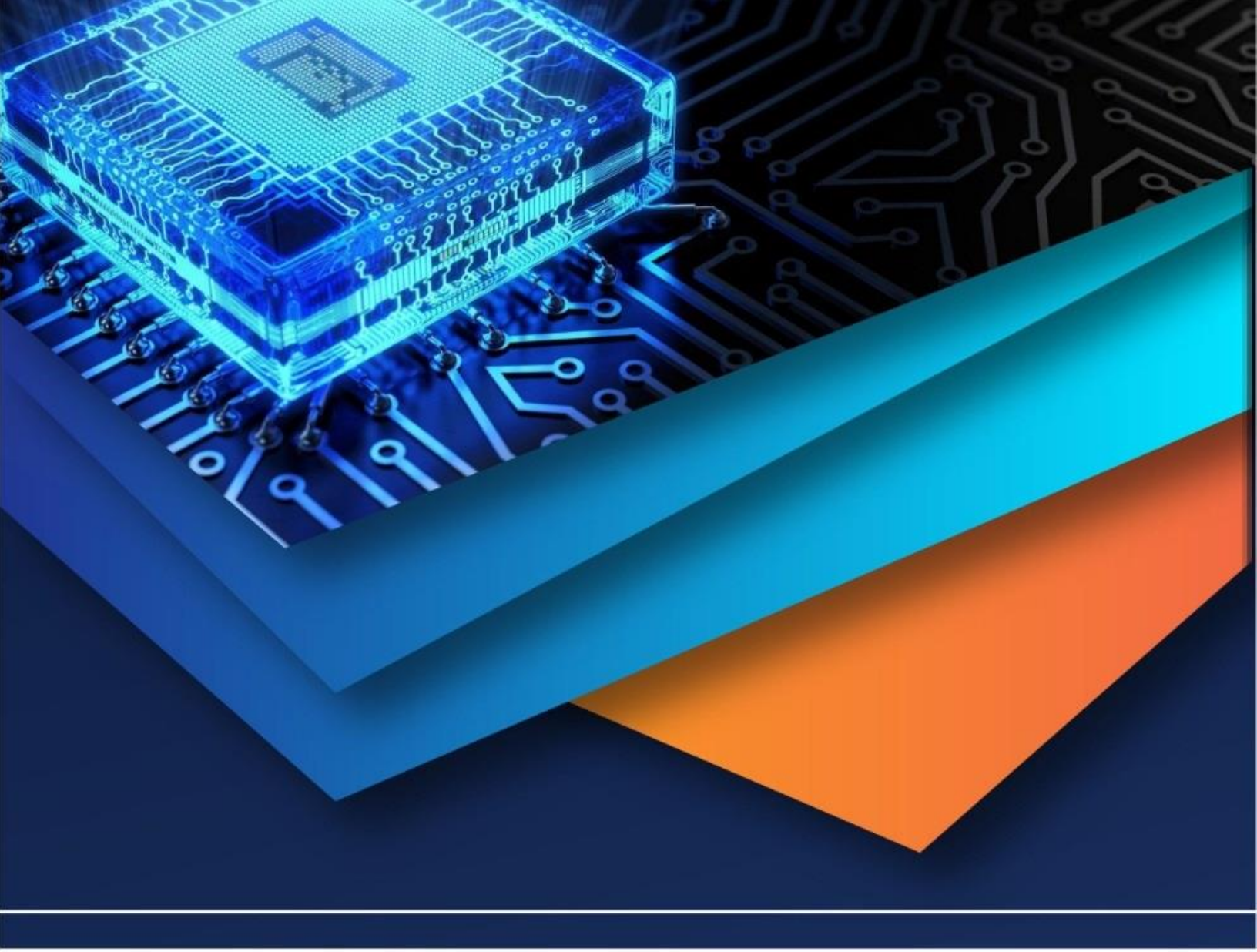
The present investigation was undertaken to analyze the diversity among popular Citrus species found in Assam. The weights of DNA was found to be 4000 bp in case of C.medica followed by C. maxima with molecular weight ~3800 bp .C. limon and C. limetta showed similar sized DNA .i.e ~3700 bp.C. sinensis showed a band size of ~3500.C.auratiifolia showed the lowest molecular weight of ~3200bp.Restriction digestion using the restriction enzymes yielded separate bands of different molecular weights implying their inter specific diversity. The findings of the present study will help in future for various studies related to analysis of numerous molecular aspects such as gene mapping, genetic fingerprinting, population studies and phylogenetic analyses.

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