# Detection of Genetic Variation in Tissue Culture Clones of Date Palm using ISSR Markers

**Thummar V. D[1] Rukam S. Tomar[2] Parakhia M. V.[3] Padhiyar S. M.[4] Rathod P. J[5]**
[1,2,3,4,5]Department of Biotechnology
[1,2,3,4,5]Junagadh Agricultural University, Junagadh, Gujarat, India

*Abstract*— Date palm is a plant having high nutritional value and long life (yielding up to 100 years). Phoenix dactylifera requires 2-5 males for pollination of 100 females' plant depending up on genetic and environment factors. Therefore paternity variation expected to very low according to PCR based techniques, Even though we have tried to find out genetic variation among tissue culture cloned plant. Tissue culture technique can be used for genetic improvement of date palm. The main purpose of this study was to evaluate the genetic variation in the tissue culture clones of date palm by using ISSR primers among mother and it's two clones. The plant DNA was extracted and subjected to detection of genetic variation in two groups of date palm using ISSR primers. In this study ISSR primers produced monomorphic bands within group-1 and group-2. Genetic variation in tissue culture clones of date palm was not detecte by UBC primer series.

***Key words:*** Date palm, clones, ISSR

## I. INTRODUCTION

Date palm Phoenix dactylifera is one of the oldest cultivated fruit trees on the earth. For dry arid regions, it is one of the most potential fruit crops [6]. The origin of the date palm can be believed to the region stretching from West Pakistan through Iran, Iraq and Arabia to Northern Africa. The western boarder of Gujarat is the only commercial cultivar of date palm in India. In Kutch there are more than 2 million date palms, the majority of them grown from seeds and offshoots, providing a huge biodiversity for experimentation. 70-80% of date palm cultivation in the coastal belt of Kutch from Anjar to Mandvi, originates from seed and the majority of fruits are of inferior quality [8]. Slow rate of date palm's vegetative propagation is the major problem in date palm cultivation. Seeds do not produce true progeny and half of seeds become useless for fruit production, because they turn out to be male. Tissue culture technique can be used for mass propagation, thus enabling rapid coverage under improved high yielding varieties. Choosing an effective method to assess genetic variability in a tissue culture group of individuals is of great interest to many researchers studying population genetics. In recent years, different molecular markers based on PCR amplification have been developed and rapidly have become essential tools in this field. For plants, ISSR has been proven to be a simple and reliable marker system with highly reproducible results and copious in polymorphisms [2]. ISSR markers, however, have more rigorous primer annealing conditions than RAPDs, which leads to superior reproducibility. Those features, along with the cost, have brought attention to these markers. The main objective of this work is to detect genetic variation in mother date palm and its two clones using ISSR markers.

## II. MATERIAL AND METHOD

### A. Plant Materials:

There are two groups of date palm. First Group has mother palm plant (M1) and its two clones (N1&N2) while second group has also one mother palm plant (M2) and its two clones (N3&N4). Young leaves of both the groups of date palm were collected from Gujarat, India. Three to five young leaves were collected and washed with dH2O and then subjected to DNA extraction.

### B. Genomic DNA Isolation from leaves:

Total DNA extraction from leaf was performed using CTAB method [1,9]. One gram of leaf was ground in liquid nitrogen using mortal and pestle and mixed with 2 ml of CTAB buffer [100 mM Tris–HCl pH 8, 1.4 M NaCl, 20 mM EDTA, 2% (w/v) CTAB, 1% (w/v) PVP, 0.2% (v/v) b-mercaptoethanol]. Extracts were collected in 2 ml eppendorf tubes and incubated at 65 ˚C for 1 hr, centrifuged at 10,000 rpm for 10 min to remove cell debris. After centrifugation supernatants were collected in 2 ml fresh eppendorf tubes, mixed with an equal volume of chloroform-isoamyl alcohol (24:1 v/v) and centrifuged at 10,000 rpm for 10 min. The aqueous phase was extracted twice with chloroform-isoamyl alcohol, recovered and mixed with two-third volume of isopropanol and keep at -80 ˚C for 2 hours. Precipitated DNA was recovered as pellet by centrifugation at 12,000 rpm for 20 min, washed with 200ul of 70% ethanol, dried and resuspended in 100ul of TE buffer (10 mM Tris– HCl pH 8, 1 mM EDTA pH 8). Extracted DNA was diluted to 1 µl: 10 µl TE buffer and used for PCR amplification. DNA quantification was done using a Picodrop. Unknown concentration was estimated by adding 10 µl of DNA sample. Each sample was diluted up to 50 ng /µl with TE buffer (10 mM Tris–HCl, pH 8.0 and 0.1 mM EDTA, pH 8.0) and stored at 4°C for further use.

### C. ISSR- PCR Reactions and Electrophoresis:

For the determination of genetic variation among mother and it's two clones, ISSR reaction was carried out in both groups of date palm. For first group 9 primers (UBC-823,825,836,843,888,890,895,897,900) were used, while for second group 10 primers (UBC-836,841,844,847,848,849,850,880,888,890) were used (Table-1). Inter simple sequence repeats (ISSR) technique was carried out according to procedure described by Martins-Lopes et al [5]. The ISSR amplification reactions were carried out in 20µl per tube, containing 1µof the plant DNA (50 ng/ µl), 0.3 µl of 1 unite Taq DNA polymerase enzyme (Invitrogen), 2.0µl 10X buffer, 0.5 µl MgCl2, 0.06µl of dNTPs (2.5 mM), 2 µl primer (10pmol/ 20 µl ). The volume was made to 20 µl with sterile distilled water . PCR tubes containing the above components were capped and given a plus spin to allow proper mixing of the reaction mixture. The tubes were then placed in Thermal Cycler

 

(Veriti, Life Technology) for amplification. Thermo-cycling conditions were as follows: an initial denaturation step of 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, a primer annealing step at 46°C for 45s, and an extension at 72°C for 2 min; then a final extension was carried out at 72°C for 5 min. The annealing temperature varied according to the melting temperature of each primer. After completion of PCR amplification, 2.5 µl of loading dye (6X) was added to each PCR tube. Samples were loaded in 1.5 % agarose gel and electrophoresis at 110 V for 1.5-2.0 hours. The gels were stained with ethidium bromide. The resolved amplification products were visualized by illumination under UV light in Gel document system. Fragment size was estimated by using a 100 base par molecular size ladder (Genetix, India).

## III. Results and Discussion

The use of molecular markers, revealing polymorphism at the DNA level, plays an important role in determination of genetic variation in plant tissue culture. In this work, the utility of ISSR markers for genetic variation of date palm was studied.

### *A. ISSR Banding Pattern for First Group*

First group of date palm (M1, N1 & N2) obtained from the Kutch region was amplified using 9 ISSR primers (Figure:-1). Out of 9 ISSR primers all primers gave reproducible amplification products. The size of the amplification product ranged from 200 to 1700 bp. The present result of ISSR showed no polymorphism, all the bands were monomorphic. Genetic variation was not detected in Mother and it's two clones of first group.

### *B. ISSR Banding Pattern for Second Group*

Second group of date palm (M2, N3 & N4) obtained from the Kutch region was amplified using 10 ISSR primers (Figure-2). Second group of date palm (M1, N1 & N2) obtained from the Kutch region was amplified using 10 ISSR primers (Figure-1). Out of 10 ISSR primers all primers gave reproducible amplification products. The size of the amplification product ranged from 200 to 1700 bp. The present result of ISSR showed no polymorphism,all the bands were monomorphic. Genetic variation was not detected in mother and its two clones of second group.

Variation in chromosome numbers and structures is possible among regenerated somaclones [3,4,7]. Inter-simple sequence repeat (ISSR) markers have been used to study the genetic variability in micro propagated fruit crops. In plantlets of almond (Prunus dulcis), regenerated by auxiliary branching, genetic stability was analyzed with RAPD markers and confirmed by ISSR analysis [10].

## IV. Conclusion

The results of genetic variation detection in somaclones and original date palm plants, with ISSR primers, showed no genotypic differences; the date palm cultivar manifested the highest genetic stability in the in vitro culture. ISSR can also be used successful for detection of somaclonal variation in cloned plants with specific purpose.

## V. References

[1] Doyle, J.J., Doyle, J.L.: Phytochemical Bulletin., 19: 11-15(1987).
[2] Gonzales, N., Knight, G., Morgan-Lopez, A., Sanenz, D., Sirolli, A.: Current research and future directions.West Port, CT: Praeger; 45–76(2002).
[3] Hao, Y. J., Deng, X.X.: In Vitro Cellular Developmental Biology Plant., 38:472-476(2002).
[4] Larkin, P., Scowcroft, W.: Theoretical and Applied Genetics., 60: 197-214 (1981).
[5] Martins-Lopes, P., Lima-Brito, J., Gomes, S., Meirinhos, J., Santos, L. & Guedes-Pinto, H.: Genetic Resource and Crop Evolution ., 54: 117-128(2007).
[6] Munier, P. 1973. Le palmier-dattier. Paris: Maisonneuve et Larose
[7] Mujib, A., Banerjee, S., Dev Ghosh, P.: Propagation of Ornamental Plants., 7:169-174 (2007).
[8] Ramdevputra, M. V., Butani, A. M., Savalia, J. J., Pansuria, A. G. and Kanzaria, D. R.: Asian J. Hort., 4(1):181-183(2009).
[9] Rathod Pankajkumar J. Biochemical and Molecular Aspects of Wilt in Chickpea: (Fusarium oxysporum f. sp. ciceri) IN CHICKPEA (Cicer arietinum L.) ISBN : 9783848425211
[10] Sarmento, D., Martins, M., Oliveira, M.M.: Options Méditerranéennes, Série A., 63: 391-395 (2005).

| Sr. No. | Primer | Sequence 5' – 3' | Tm ($^0$C) |
|---|---|---|---|
| 1 | UBC-823 | TCTCTCTCTCTCTCTCC | 46.0$^0$C |
| 2 | UBC-825 | ACACACACACACACACT | 46.0$^0$C |
| 3 | UBC-836 | AGAGAGAGAGAGAGAGYA | 46.0$^0$C |
| 4 | UBC-841 | GAGAGAGAGAGAGAGAYT | 46.0$^0$C |
| 5 | UBC-843 | CTCTCTCTCTCTCTCTRA | 46.0$^0$C |
| 6 | UBC-844 | CTCTCTCTCTCTCTCTRC | 46.0$^0$C |
| 7 | UBC-847 | CACACACACACACACARC | 46.0$^0$C |
| 8 | UBC-848 | CACACACACACACACARG | 46.0$^0$C |
| 9 | UBC-849 | GTGTGTGTGTGTGTGTYA | 46.0$^0$C |
| 10 | UBC-850 | GTGTGTGTGTGTGTGTYC | 46.0$^0$C |
| 11 | UBC-880 | GGAGAGGAGAGGAGA | 46.0$^0$C |
| 12 | UBC-888 | BDBCACACACACACACA | 46.0$^0$C |
| 13 | UBC-890 | VHVGTGTGTGTGTGTGT | 46.0$^0$C |
| 14 | UBC-895 | AGAGTTGGTAGCTCTTGATC | 46.0$^0$C |
| 15 | UBC-897 | CCGACTCGAGNNNNNNATGTGG | 46.0$^0$C |
| 16 | UBC-900 | ACTTCCCCACAGGTTAACACA | 46.0$^0$C |

Table 1: List of ISSR primers (Sigma aldrich, India) used for detection of genetic variation in date palm

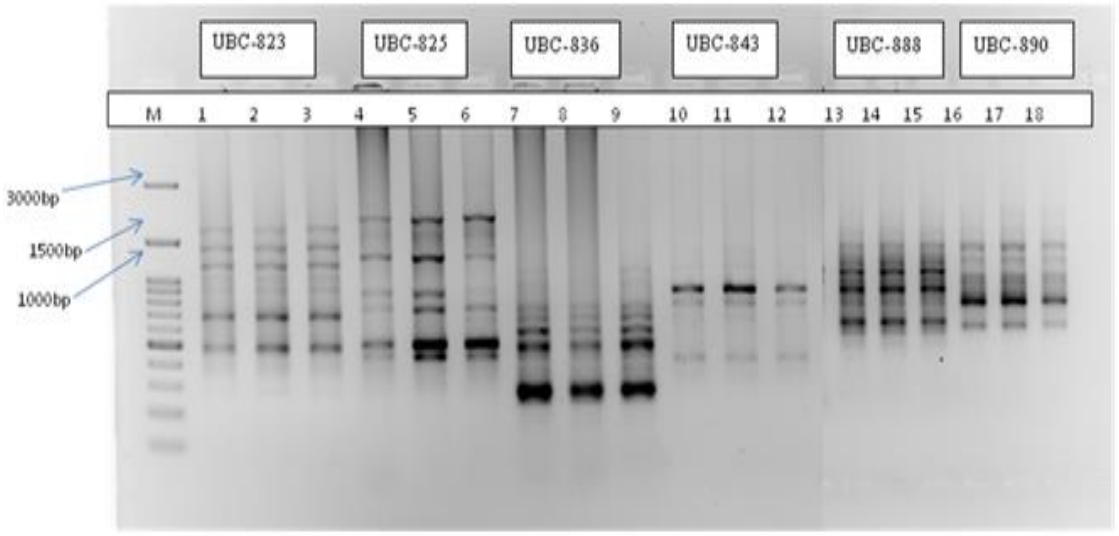


Fig. 1(A):

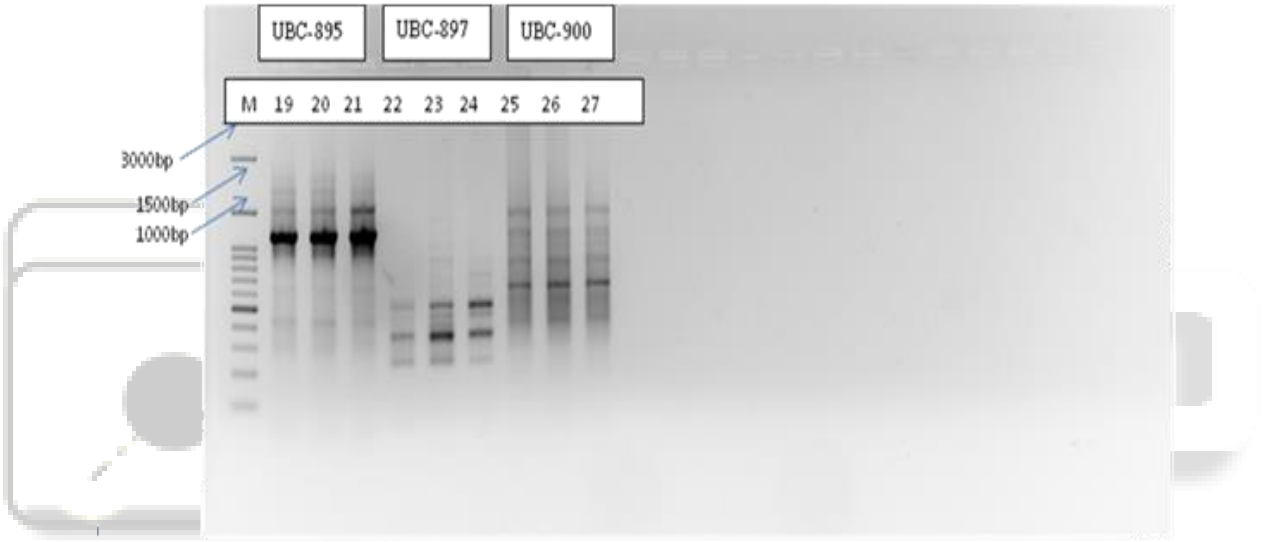


Fig. 1: (A)(B) 9 Inter Small Sequence Repeats (ISSR) amplification pattern obtained for DNA of mother plant, M1(lane 1,4,7,10,13,16,19,22,25) and it's two micropropagated clones N1(lane 2,5,8,11,14,17,20,23,26) & N2 (lane 3,6,9,12,15,18,21,24,27) of group-1. M: 100bp DNA ladder.

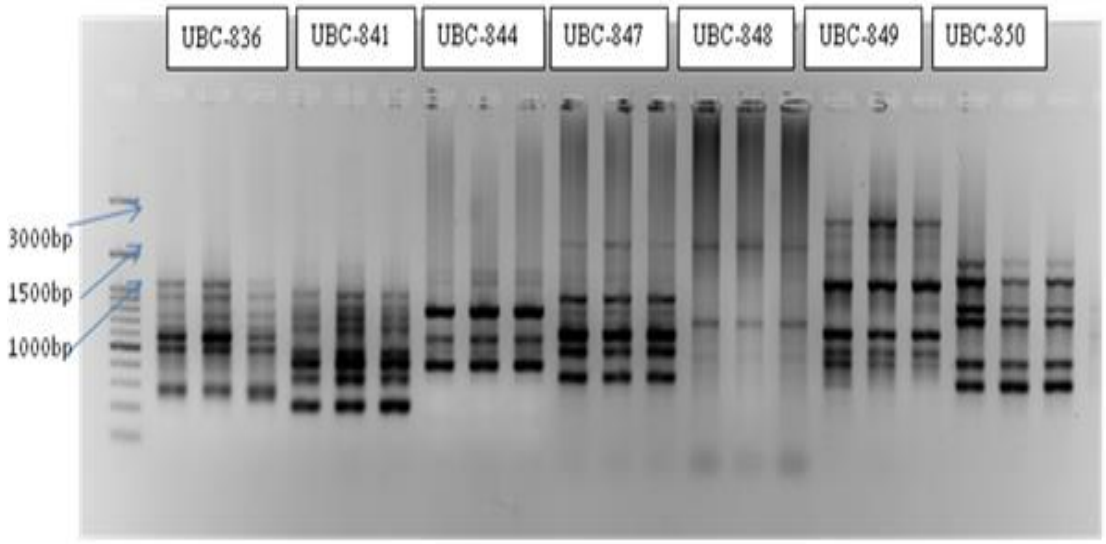


Fig. 2(A):

 

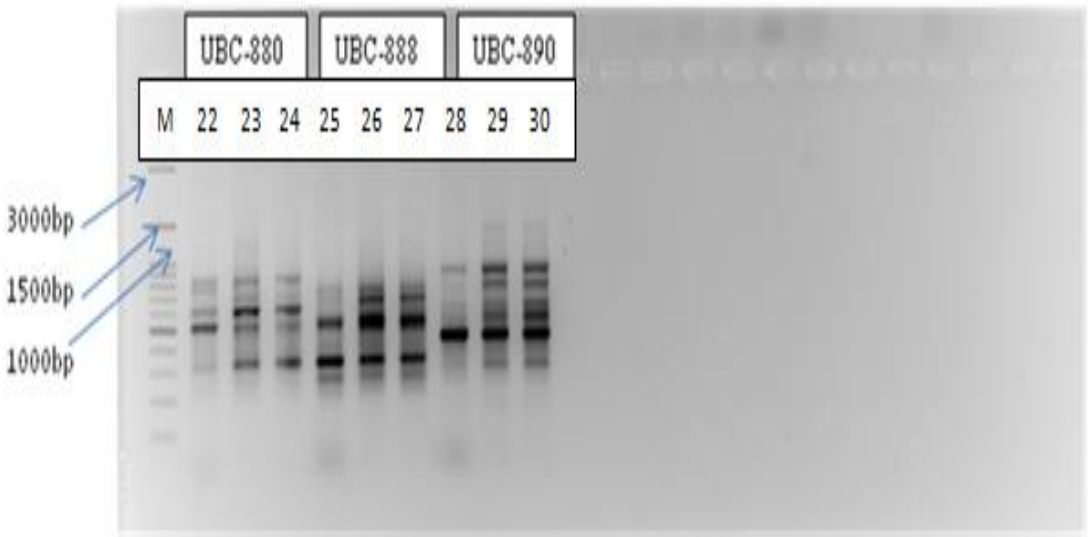


Fig. 2(A)(B): 10 Inter Small Sequence Repeats (ISSR) amplification pattern obtained for DNA of mother plant, M2 (lane 1,4,7,10,13,16,19,22,25,28) and it's two micro propagated clones N3(lane 2,5,8,11,14,17,20,23,26,29) & N4 (lane 3,6,9,12,15,18,21,24,27,30) of group-1. M: 100bp DNA ladder.

 