Agroforestry *Newsletter*

National Research Centre For Agroforestry, Jhansi-284 003

Vol.19, No. (3) **July- September, 2007**

Development of DNA Isolation Protocol for Polymerase Chain Reaction Based Marker-Assisted Breeding in *Jatropha curcas* L.

Jatropha curcas L., family Euphorbiaceae, is native of Mexico and tropical South America. In India, it is commonly known as physic nut, purging nut, ratanjyot, chandrajyot, jangli arandi, kala aranda etc. and mainly distributed in states like Rajasthan, Madhya Pradesh, Chhatishgarh, Maharashtra, Gujarat, Andhra Pradesh and Tamilnadu. It is very hardy in nature, can grow in poor soils with annual rainfall of 300-1000 mm. and withstand temperature up to 45°C. It is easily propagated through seeds as well as vegetative part. It can be grown as block plantation, boundary/bund plantation, filler plantation or with intercrops. For the genetic improvement of this species, assessment of genetic variability in the population is pre requisite .For the purpose, molecular markers are the best tools because they permit the analysis of diversity at the molecular level.

An important, but often limiting step in marker-assisted breeding is the efficient isolation of DNA for polymerase chain reaction (PCR) amplification. Isolating genomic DNA from the leaves of *Jatropha curcas* is problematic because of the presence of fiber, phenols and other organic compounds, which act as inhibitors. Initially several DNA extraction protocols as well as commercial preparation kit (Quaigen and Axygen) were employed, but the quality and quantity of the DNA obtained was not satisfactory. In some of the procedures, DNA was extracted but procedure required large volumes of reagents and took a long time to prepare numerous samples which is very expensive affair as well as time consuming. A protocol to extract DNA was developed using CTAB methods. This protocol requires minimal amounts of reagents and plastic ware, allowing for the simultaneous preparation of 30-40 samples, totaling up to 80 samples per day. To further purify the DNA, the final precipitation step is repeated 3 times before resuspending the DNA in Tris-EDTA. This repetition increases restriction enzyme digestion, making the use of the isolated DNA in RAPD, RFLP and AFLP techniques.

The isolated DNA was quantified spectrophotometrically, with yields ranging from 70-150 µg per gram of tissue. The A260/A280 ratio was 1.64-2.05 among the first group of 12 samples analyzed, indicating clean DNA. RNA contamination, if any, can be removed by digesting the sample with RNAse (20 µg/mL), followed by phenol extraction and precipitation.

For the extraction of DNA, leaves of *Jatropha curcas* were used. The materials were taken from the repository at National Research Centre of Agroforestry, Jhansi.

Solution and buffers for *Jatropha curcas* DNA isolation:

1. 0.5 M EDTA pH 8.0 : Dissolved 37.22g EDTA disodium salt and 4.0g Sodium hydroxide in 150 ml H_2O and adjusted the pH by NAOH solution. Final volume was made up to 200 ml.

2. 1M Tris-Cl pH 8.0: Dissolved 24.33 g Tris base in 150 ml distilled water. Adjusted to the pH 8.0 by HCL and made up final volume to 200ml with water.

3. 5M NaCl: Dissolved 58.44g NaCl in distilled water and made up final volume to 200 ml with water.

4. CTAB (10%) W/V: Dissolved 200g CTAB in 180 ml distilled water. CTAB was dissolved by warming the solution. The final volume was made up to 200ml

5. TE Buffer pH 8.0: 2ml Tris-Cl from 1M Tris-Cl stock and 0.04ml EDTA from 0.5M EDTA stock were taken. The final volume was made up to 200 ml.

6. Tris Borate EDTA buffer or TBE buffer pH 8.0: Dissolved 27g Tris base, 13.75g boric acid, 10ml (0.5M) EDTA in 400ml distilled water and made final volume to 500 ml with water.

7. CTAB total DNA Extraction Buffer:

Final concentration	**Stock concentration**
20mM EDTA	8ml (0.5M EDTA)
100mM Tris-Cl	20ml(1M Tris-Cl pH 8.0)
1.4M NaCl	56 ml (5MNaCl)
2% W/V CTAB	40ml (10% CTAB)

Mixed and volume made up to 200ml with water.

8. Loading dye (Bromophenol blue) :

Final concentration	**Stock concentration**
0.1M EDTA	10ml (0.5 M)
40% Sucrose	20g
25% Bromophenol blue	125mg

Final volume was made up to 50ml with distilled water.

DNA Extraction protocol:

1. Grind 0.25 g of leaf tissue to a fine powder using a mortar, pestle and liquid nitrogen. Transfer the powder to an Eppendorf tube. Add 500 µL extraction buffer .
2. Vortex the mixture. Incubate in a water bath at 65ºC for 1hr.
3. Add 500 µL ultra pure chloroform. Mix thoroughly till the bottom becomes green.
4. Centrifuge at 6000 rpm for 10 min.
5. Transfer the supernatant to a clean Eppendorf tube. Add 0.6% µL cold isopropanol. Incubate on ice for 1hr.
6. Centrifuge at 4000rpm for 10 min. Discard the supernatant. Wash the pellet with 300 µL 70% ethanol and let dry.
7. Resuspend pellet in 600 µL TE buffer containing RNAse.
8. Add 300 µL cold ethanol. Incubate on –20 ^{0}C for 1hr.
9. Centrifuge at 6000rpm for 10 min. Wash the pellet with 300 µL 70% ethanol and let dry.

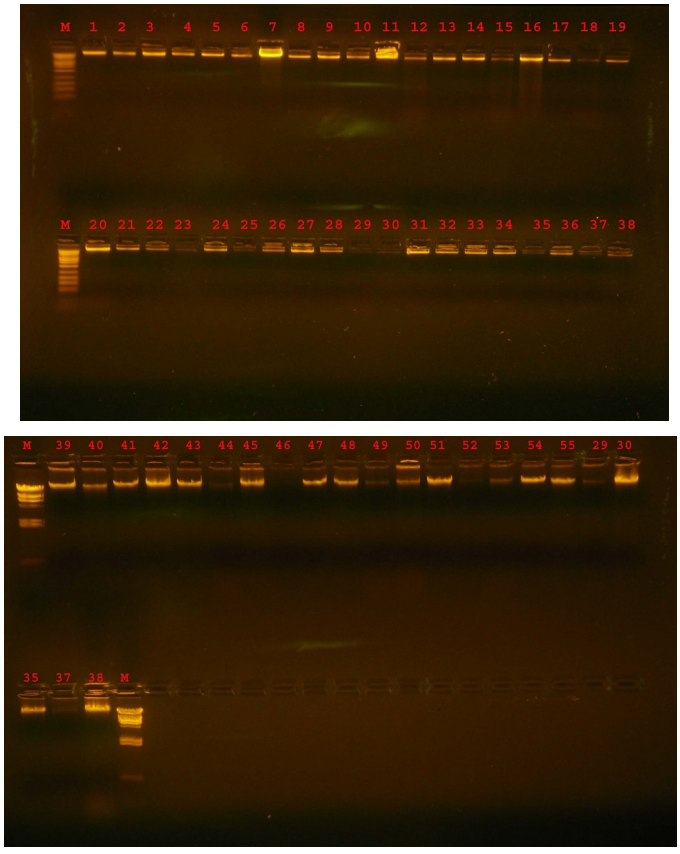

Figure. DNA isolated from different *Jatropha curcas* germplasm

10. Resuspend the pellet in 300 µL TE buffer. Quantify the DNA spectrophotometrically at 260 nm.
11. Electrophorese in agarose gel to verify the DNA quality and quantity (Figure.).

This protocol allows for simultaneous DNA extraction from numerous samples and

uses small volumes of reagents while producing clean and high-quality DNA that is suitable for RAPD, RFLP and AFLP techniques. The protocol can be applicable to other tropical and subtropical tree species.

R.V. Kumar, Y.K. Tripathi, V.P. Yadav, S.P. Ahlawat and V.K. Gupta
National Research Centre for Agroforestry, Jhansi

INSTITUTE RESEARCH COUNCIL

XVIII th Institute Research Council (IRC) meeting was held on 4th to 6th July, 2007. All the scientists of the Centre participated in the meeting and presented the progress and significant findings of their projects. Three new projects were approved by the IRC.

CELEBRATION OF ICAR FOUNDATION DAY

Centre celebrated 78th ICAR FOUNDATION DAY on 16th July, 2007. On this occasion research activities on Agroforestry, Agrihorticulture, Silvipasture, Tree Improvement and Watershed Development were exhibited and farmers of Ganeshgarh, Karari and Garhkundar villages were also participated. Dr. V. K. Gupta (Acting Director) delivered a foundation day lecture. The event got wide coverage in press and media.

HUMAN RESOURCE DEVELOPMENT

- Dr. P. Rathakrishnan, Scientist (Forestry), Dr. C. K. Bajpai, Sr. T. O. (T-7/8) and Sh. Mahendra Kumar, Sr. Clerk of the Centre participated in the Hindi Training on " Gahan Hindi Training and Workshop" from 17- 21 July, 2007 at NAARM, Hyderabad.
- Sh. Rajendra Singh, Tech. Officer participated in the Training on "E. Learning" from 20-25 August, 2007 NAARM, Hyderabad.
- Sh. R. H. Rizvi, Scientist (Comp. Appli.) and Sh. B. S. Tomar, Sr. Clerk participated in training on "Intelligent Reporting System (IRS) from 30 to 31, August,2007 at NAARM, Hyderabad.

INSTITUTE JOINT STAFF COUNCIL

Institute Joint Staff Council meeting was held on 14th August 2007 under the Chairmanship of Director Dr. S. K. Dhyani.

SADBHAWNA DIVAS

Centre observed SADBHAWNA DIVAS on 20th August 2007. On this occasion all the Scientists, Officers and other staff members of the Centre took Oath to inculcate *Sadbhawna* in their acts and behaviour.

SC/ST CELL

The SC/ST Cell has been constituted to (i) Ensure compliance of office orders of reservation issued from time to time in favour of SC/ ST employees and (ii) assist the Liaison Officer to discharge his duties effectively. The committee is as under:

1. Dr. Ram Newaj, Sr. Scientist - Liaison Officer & Chairman

2. Dr. S. P. Ahlawat, Sr. Scientist
3. Dr. A. Venkatesh, Sr. Scientist
4. Sh. Birendra Singh, Sr. Clerk
5. Sh. D. K. Awasthi, AAO

WOMEN CELL

As per the guidelines of the ICAR, New Delhi, and norms laid down by the Hon' ble Supreme Court, a Women Cell has been constituted at the Centre as under:

1. Smt Uma, Tech. Officer, NRCAF, Jhansi & Chairman,
2. Km. Divya Sharma, Professional –I, Development Alternatives (NGO), Taragram, Orchha, Tikamgarh (M.P.)
3. Smt. Kaushlya Devi, Jr. Clerk, NRCAF, Jhansi.

PME MEETING

PME meeting was held on 5th and 6th September 2007. Agroforestry concerns in relation to global warming were discussed amongst the Scientists. All the Scientists participated in the PME meeting. Contingent action plan for different ongoing experiments was discussed in the light of preventing drought in the region.

QUINQUENNIAL REVIEW TEAM (QRT)

Under the chairmanship of Dr. R. P. Awasthi, Former Vice Chancellor, Dr.Y. S. P. U. H. & F, Solan (H. P.) third QRT (2002-2006) team visited the AICRPAF centers (PAU, Ludhiana; HAU, Hisar; UAS, Dharwad; BSKV, Dapoli and MPKV, Rahuri) from 07 to 29 July, 2007 and JNKVV, Jabalpur from 07 to 09 September, 2007 to review the work under the project. Dr. R. P. Awasthi, (Chairman QRT) along with other team members visited the Centre from 10 to 13 September, 2007 for interaction with the Scientist and officers of the Centre. Dr. A.K. Gogoi, ADG, (Ag/AF.) also participated in the interaction meeting.

CENTRAL JOINT STAFF COUNCIL

Sh. B. S. Tomar, CJSC Member of the Centre, participated in the 26th Annual Meeting of CJSC from 13.9.2007 to 16.9.2007 at NAARM, Hyderabad.

RETIREMENT

Sh. R. N. Singh, Tech. Officer (T-5) of the Centre retired on 31st July, 2007. The staff members bid a grand farewell to the officer.

BER BUDDING TRAINING PROGRAMME

Centre organized Ber Budding Training from 28-30 July, 2007 at village Garh Kundar in Tikamgarh (M.P.). This programme benefited about 37 farmers. Ber variety Banarsi karaka is perpetuated on farmers field.

ENGINEER DAY OBSERVED

Centre observed Engineer's Day (Shi Vishkarma Jayanti) on 16th September, 2007.

VISITORS

1. Dr. R.P.Awasthi, Ex. V C& Chairman QRT, Dr.YSPUH& F, Solan (H. P.).
2. Dr. A. K. Gogoi, ADG (Agro./AF), ICAR, KA B-II, New Delhi-110114.
3. Dr. V. K. Mishra, Ex-Dean & Head, Department of Forestry, Solan (H.P.).
4. Dr. A.K. Mandal, Director, Tropical Forest Research Institute, Jabalpur (MP).
5. Dr. U. C. Sharma, Ex-National Coordinator, NATP, Jammu (J&K).

NEW EXTERNALLY AIDED PROJECTS

	Name of the Project	**Funding Agency**	**Duration of the Project**	**Principal Investigator**
1	Development of Bamboo Based Agroforestry Systems for Six Agroclimatic Zones	National Bamboo Mission, Department of Agriculture and Cooperation, Ministry of Agricultre, GOI, New Delhi	1 September 2007 to 31 August 2010	Dr. S.P. Ahlawat, Sr. Scientist
2	Spatial and Temporal Analysis of Agroforestry Interventions in North –Western India using GIS and Remot Sensing	DST, Ministry of Science and Technologies, New Delhi	1October 2007 to 30 September 2010	Dr. R. H. Rizvi, Scientist (Sr. Scale)

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EXHIBITION STALL

Centre participated in Foundation day of Indian Institute of Vegetable Research (IIVR), Varanasi (U.P.) on 28th September 2007. Hon'ble Dr. Mangala Rai, D.G., DDG (Horti.), ICAR, New Delhi and Ex. V. C. of Purvanchal University visited the Exhibition Stall of NRCAF