



RESEARCH ARTICLE

IDENTIFICATION OF ELITE BIOTYPE OF *TERMINALIA ARJUNA* USING RAPD MOLECULAR MARKER FOR PRODUCTION OF HIGH YIELD TASAR COCOONS

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ARTICLE INFO

Article History:

Received 14th, January, 2015

Received in revised form 23th, January, 2015

Accepted 13th, February, 2015

Published online 28th, February, 2015

Key words:

T. arjuna, AGE, RAPD primers, PCR, Gel Doc, UPGMA.

ABSTRACT

Advance of molecular approach and biochemical studies, we identify elite biotypes of *Terminalia arjuna* which are grown in local area of Telangana region. Using five RAPD primers only one primer viz. OPP-3 produced unique DNA bands in 8 genotypes of *T. arjuna*, which could be used as differential marker for identification elite biotype in *T. arjuna*. We identified the elite biotype of Jakaram-FDA with unique band (2500, 2000 bp) and produce maximum protein content (369 µl/5gm), while this genotypes produced large size cocoon (19.27±0.9 gm). Minimum amount of protein was observed in Eturnagaram-FDA (206 µl/5gm) & produced small size cocoon (4.9±0.14 gm). Using UPGMA method dendrogram was constructed by OPP-3 primer showed that 10 genotypes of *T. arjuna* grouped into two main clusters. First cluster revealed 75% and second cluster revealed 65% genetic similarity. This method is useful to conserve elite biotype (high yield), which producing quantitative and qualitative cocoon, which are used in silk industry.

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INTRODUCTION

T. arjuna belongs to family Combretaceae, in this family approximately 200 species are distributed around tropical & sub tropical regions. Srivastav *et al.*, (2003) reported that 24 *Terminalia* species are present in various parts of India. Sericulture is a small industry for making the silk and its products. *Antheraea mylitta* (Drury) depends on primary host, such as *T. arjuna*, *T. bellirica*, *T. chebula* etc, for feeding Renuka *et al.*, (2013). The adult stages of *Antheraea mylitta* required *T. arjuna* to complete their life cycle Renuka *et al.* (2013). Thus *Terminalia* genera are known as backbone of the Tasar silk industry Sarawat *et al.* (2011) The plant *T. arjuna* is used in medical system as Ayurvedic medicine which play important role in cordiatonic (Tripathi *et al.*, 1996). The bark of *T. arjuna* used in treatment of cancer (Pettit *et al.*, 1996) Diabetic Manonmani *et al.*, (2002) used in coronary artery disease, heart failure hypercholesterole, rolemia Sarwat *et al.*, (2006) reported that bark is useful in treatment related to Urinary discharges.

Majority of tribes Nayakpod, Koya's are conducting the silk rearing in different regions of Venkatapur & Jakaram areas of Warangal district, Mahadevpur of Karimnagar district and Jannaram of Adilabad districts in Telangana State. Tribes are depends on the forest products for their livelihood such as timber, ayurvedic & unani medicine from ancient days to still today. In their livelihood the silk rearing plays a vital role for survival and economical benefit. They rear silk two times per annum,

sometimes may rear three times per annum if more favorable conditions. However farmers don't have adequate knowledge for pruning the plant, farm disease, developing of large size cocoon, quality of cocoon which brings low returns for their selection. Identification of best biotypes of *T. arjuna* is needed to gain good yield of cocoon and silk. Primary metabolites of *T. arjuna* is responsible for growth, protein content, development of large size cocoons and used in development of pharmaceutical compounds.

With advance of molecular approach using PCR based molecular markers such as RAPD, helpful for identification of best biotypes in *T. arjuna*. This is the first reports on genetic variation with in *T. arjuna* genotype, which are growing in local area of Telangana State, India. Similar observation such as genetic variation in tree plants were reported by Sing *et al.*, 1999; Belaj *et al.*, 2002; Shah *et al.*, 2005; Sarwat *et al.*, 2010 and also to estimate protein content in *T. arjuna*. Protein estimation in *T. arjuna* were reported by Rishi *et al.*, 2009; Sagwan *et al.*, 2010; Talreja, 2011 & Yadav *et al.*, 2012. In the present study we have develop RAPD molecular markers to discriminate the elite biotypes of *T. arjuna* and protein estimation, which are grown in local area of Telangana State.

MATERIAL AND METHODS

Different genotypes of *T. arjuna* were obtained from various districts (Adilabad, Karimnagar and Warangal) of Telangana State as shown in table.1.

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Estimation of Proteins

Total leaf protein was estimation in 10 genotypes of *T. arjuna* by the method of Sharma et.al., (2012).

Low level of Genomic DNA extraction

Genomic DNA was extracted from young leaves of the above mentioned (Rogers & Benedict, 1985). Briefly, 1.5 g of leaves was ground with a pre-cold treated pestle and mortar in 2500 µl extraction solution (38 mM EDTA, 1.7 M NaCl), (pH 8.0), 80 mM Tris-HCl (pH 8.0) and 2% mercaptoethanol). The sample was incubated at 62°C for 45 min. After incubation, the sample was cooled to room temperature and chloroform and isoamyl alcohol (24:1) was added, centrifuged at 8000 rpm for 10 min. Collected the supernatant and mixed with equal volume of cold isopropyl alcohol and incubated for 15 min at -20°C. After centrifugation, the pellet was dried, washed and dissolved in 1% TE buffer and was used in RAPD- PCR amplification experiments.

RAPD-PCR amplification

RAPD-PCR amplification was performed by the modification method of Vishal et.al., (2009). The PCR reactions were setup in a 20 µl (2 µl of 40 ng genomic DNA of Young leaves, 5 µl of 0.4mM dNTPs (Fermentos, USA), 4 µl of 2.5mM MgCl₂, 5µl of each primer (20 pmol/µl), 1X PCR reaction buffer (20mM Tris hydrogen chloride, pH 8.2, 50 mM Potassium chloride and 0.3% gelatin), 3 µl of PCR grade water & 1µl of 0.2 unit of Taq DNA polymerase was used.

Amplification was performed on a thermal cycler (Model No. TC-3000 Techne, USA) with a program initial denaturation temperature 94° C for 8 min, followed by 38 cycles of 94 ° C for 50 sec, 32 ° C for 40 sec and 72 ° C for 1 min followed by final extension temperature at 72° C for 10 min and separate the sample under AGE electrophoresis.

Separation of amplicon on AGE

The obtained amplification PCR products were separated on 1.0% of a Agarose gel, in 1.5 X TE buffer at 64 V for 1 hrs. amplicons size were estimated by using 2 Kb DNA ladder (Fermentos, USA). DNA amplicons were visualized in gel documentation system (Bio-Rad, USA).

Dendrogram analysis

Dendrogram was constructed in 10 genotypes of *T. arjuna*, to study genetic similarity using Nei and Li, 1979 method.

Table 2 RAPD-PCR primers used for identification of elite biotype in 10 genotype of *T.arjuna*

S.No	Primer Code	Base Sequence	Annealing temperature (°C)
1	OPP-01	GTAGCACTCC	32
2	OPP-03	GTGATACGCC	32
3	OPAP-04	CTCTTGGGCT	32
4	OPAP-05	GACTTCAGGG	33
5	OPW-18	TTCAGGGCAC	32

PCR Conditions

Table 3 the programming conditions of thermocycler for RAPD-PCR amplification in 10 genotypes of *T.arjuna*

Steps	Temp(°C)	Duration(min.)	Cycles(No.)
Initial denaturation	94	4.0	1.0
Denaturation	94	1.0	38.0
Annealing	32	1.0	38.0
Extension	72	2.0	38.0
Final extension	72	10.0	1.0

RESULT AND DISCUSSION

Protein estimation

Total leaf protein was estimated in 10 genotypes of *T. arjuna* which are grown in local region of Telangana State. Maximum amount of protein was observed in Jakaram-FDA (369 µl/5gm), minimum amount of protein was observed in Eturnagaram-FDA (206 µl/5gm), and remaining as follows Vekatapur-STRC (268 µl/5gm), Venkatapur-FDA (300 µl/5gm), Janaram-STRC (297 µl/5gm), Jakaram-STRC (310 µl/5gm), Mahadevpur-FDA (301 µl/5gm), Janaram-FDA (265 µl/5gm) & Mahadevpur-STRC (255 µl/5gm) and Laknavaram-FDA (256 µl/5gm).

RAPD PCR amplification

5 primers Random Amplified polymorphic DNA viz. OPP-1, OPP-3, OPP-4, OPAP-5, OPW-18 were utilized for PCR amplification. Small amount of genomic DNA (20 µl) isolated from young leaves of 10 genotypes of *T. arjuna* given table-1. Which are wildy grown in different districts of Telangana. Out of 5 primers only 1 primes viz. OPP-3 produced distinct scorable bands of all 10 genotypes of the total 68 distinct scorable bands. 37 bands were polymorphic. A dendrogram was constructed using genetic similarity matrix generated by OPP-3.

Dendrogram analysis

Dendrogram results showed that 10 *T. arjuna* genotypes could be grouped into two clusters. The main cluster separated at genetic similarity (75%) compressed of two sub clusters.

Table 1 Listed different genotypes of *T.arjuna* which are collected from different regions of Telangana State

S.No	Districts	Regions	Location	No. of samples collected
1	Adilabad	Jannaram	State Tasar Rearing Centers	1
2	Adilabad	Jannaram	Forest Divisional Area	1
3	Karimnagar	Mahadevpur	State Tasar Rearing Centers	1
4	Karimnagar	Mahadevpur	Forest Divisional Area	1
5	Warangal	Eturnagaram	Forest Divisional Area	1
6	Warangal	Jakaram	State Tasar Rearing Centers	1
7	Warangal	Jakaram	Forest Divisional Area	1
8	Warangal	Laknavaram	Forest Divisional Area	1
9	Warangal	Venkatapur	State Tasar Rearing Centers	1
10	Warangal	Venkatapur	Forest Divisional Area	1

Table 4 Different sizes of cocoon were produced from 10 genotypes of *T.arjuna* obtained from Adilabad, Karimnagar & Warangal districts of Telangana State, India

S.No	Districts	Regions	Location	Name of the Ecorace	Cocoon weight (Grams)
1	Adilabad	Jannaram	State Tasar Rearing Centers	Daba TV	14.13 ± 0.39
2	Adilabad	Jannaram	Forest Divisional Area	Daba TV	12.06 ± 0.48
3	Karimnagar	Mahadevpur	State Tasar Rearing Centers	Daba TV	15.38 ± 0.30
4	Karimnagar	Mahadevpur	Forest Divisional Area	Daba TV	11.13 ± 0.22
5	Warangal	Eturnagaram	Forest Divisional Area	Daba TV	4.9±0.14
6	Warangal	Jakaram	State Tasar Rearing Centers	Daba TV	14.36 ± 0.11
7	Warangal	Jakaram	Forest Divisional Area	Daba TV	19.27±0.9
8	Warangal	Laknavaram	Forest Divisional Area	Daba TV	10.41 ± 0.23
9	Warangal	Venkatapur	State Tasar Rearing Centers	Daba TV	12.19 ± 0.27
10	Warangal	Venkatapur	Forest Divisional Area	Daba TV	10.14 ± 0.11

The 1st sub cluster included State Tasar Rearing Centers of Jakaram, Venkatapur and Jannaram. The 2nd sub cluster included Forest Divisional Area of Laknavaram, Warangal Dist, Jannaram, Adilabad Dist. The second main cluster separated at genetic similarity (0.60%) of State Silk Rearing Center of Mahadevpur and Forest Divisional Area of Mahadevpur, Karimnagar Dist. The 2nd sub cluster included of Forest Divisional Area of Eturnagaram, Venkatapur of Warangal Dist. The 3rd sub cluster included Forest Divisional Area of Jakaram, Warangal Dist.

difficult because it has high phenolic compounds. We followed the modified procedure of Rogers & Benedich (1985); Dellaporta *et.al.*, (1983). Small amount of genomic DNA (20µl) was obtained and used in PCR amplification.

Five different RAPD primer viz. OPP-1, OPP-3, OPP-4, OPAP-5, OPW-18 were used for amplification of genomic DNA isolated from young leaves of 10 genotypes of *T. arjuna*. Out of 5 primers only 1 primer viz. OPP-3 produced unique bands in 8 genotypes viz. Venkatapur-STRC (380 bp), Venkatapur-FDA (120 bp), Jannaram-STRC (400bp), Jakaram-FDA (2500, 2000 bp), Jakaram-STRC (300 bp), Mahadevpur-FDA (1400, 280 bp), Jannaram-FDA (510 bp) & Mahadevpur-STRC (660 bp) and 2 genotypes viz. Eturnagaram-FDA & Laknavaram-FDA no amplification was observed. Similar work was reported and constructed genetic similarity in *Terminalia* species reported by Biswas *et.al.*, 1992; Andersen *et.al.*, 1990).

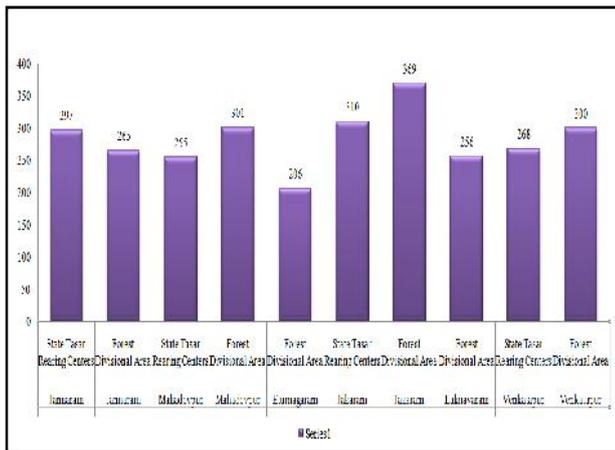


Fig. 1 Estimation of total leaf protein in 10 genotypes of *T. arjuna*.

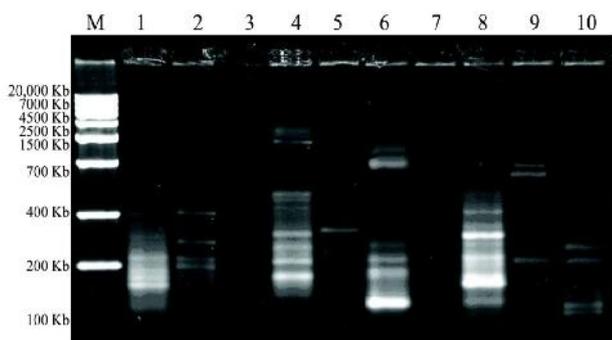


Fig. 2 RAPD profile of 10 genotypes in *T.arjuna* produced by OPP-3 primer

Fig 2. Lane M: Marker, Lane 1: Jannaram-STRC, Lane 2: Mahadevpur -STRC, Lane 3: Laknavaram-STRC, Lane 4: Venkatapur-STRC, Lane 5: Laknavaram FDA, Lane 6: Jakaram-FDA Lane 7: Jannaram -FDA, Lane 8: Jakaram-STRC, Lane 9: Venkatapur-FDA, Lane 10: Mahadevpur-FDA,

Isolation of genomic DNA and RAPD analysis was studied in 10 genotypes of *T. arjuna* which are grown in different regions of Telangana State. Isolation of genomic DNA from *T.arjun* is

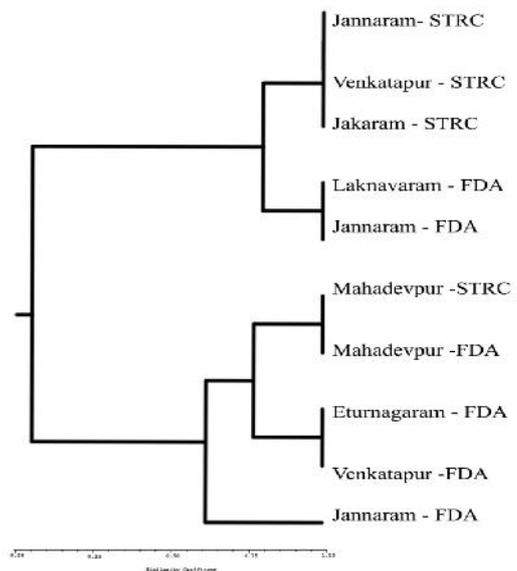


Fig.3 RAPD PCR based Dendrogram analysis in 10 genotypes of *T.arjuna*

Using UPGMA method dendrogram was constructed by OPP-3 primer showed that 10 genotypes of *T. arjuna* grouped into two main clusters. First cluster revealed 75% and second cluster revealed 65% genetic similarity. Similar work reported by Deshmukh *et.al.*, (2009) in *Terminalia* species viz. *T. catappa*, *T.arjuna*, *T.bellerica*, *T.chebula* & *T. tomentosa*.

Among 10 genotypes only 8 varieties manifested unique DNA bands, which could be used as differential marker for

identification of elite biotype in *T. arjuna*. We identified the elite biotype (Jakaram-FDA) with unique band (2500, 2000 bp) and rich in protein content (369 µl/5gm) which produces large size cocoon 19.27±0.9 gm given in table.4, while this cocoon are used in silk industry.

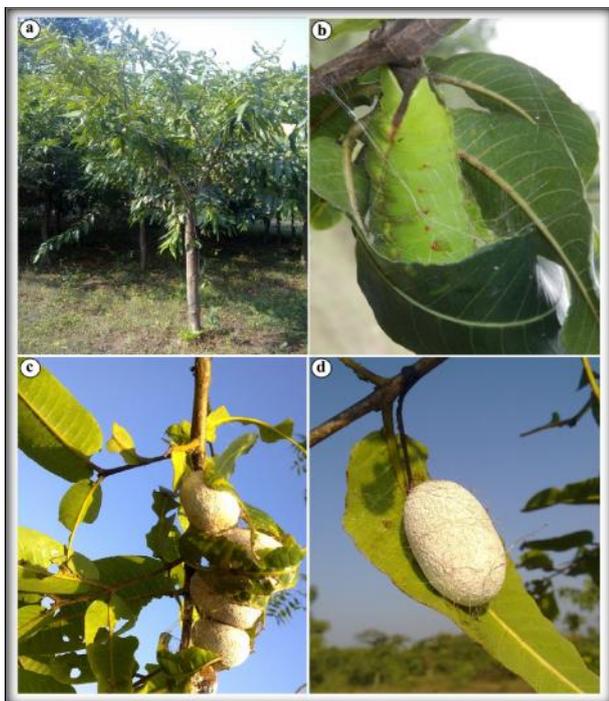


Fig 4 A. Elite biotype of *T. arjuna* (Jakaram-FDA aera). B. Spinning of Cocoon by *A. mylitta* C. Bunch of Cocoons. D. Pre harvested Cocoon.

CONCLUSION

In the present study we have developed a protocol for identification of elite biotype in *T. arjuna* genotypes was possible with molecular marker (region-specific marker). This method is useful to conserve elite biotype (high yield), which producing quantitative and qualitative cocoons are used in silk industry.

Acknowledgement

The first author acknowledge University Grants Commission(UGC) for financial assistance under Rajiv Gandhi National Fellowship.

References

1. A.L. Miller, Altern. Med. Rev. 3 (1998) 422–431.
2. Andersen, W.R. and D.J. Fairbanks, 1990. Molecular markers: important tools for plant genetic resource characterization. Diversity, 6: 51-53.
3. Belaj, A.; Satovic, Z.; Rallo, L. and Trujillo, I, 2002. Genetic diversity and relationships in olive (*Olea europaea* L.) germplasm collections as determined by randomly amplified polymorphic DNA. Theoretical and Applied Genetics, vol. 105, no. 4, p. 638-644.
4. Biswas M., Ghosh A. K. and Haldar P. K., 2010, Anti-leishmanial and anticancer activities of a pentacyclic triterpenoid isolated from the leaves of *Terminalia arjuna* (Combretaceae), Tropical J. Pharma, Research, 9(2) 135-140.
5. Dellaporta, S.L.; Wood, J. and Hicks, J.B. A plant DNA miniprep: version II. Plant Molecular Biology Reporter, March 1983, vol. 1, no. 1, p. 19-21.
6. G. Manonmani, K. Anbarasi, K. Balakrishna, G. Veluchamy, C.S. Shyamala Devi, Biomedicine 22 (2002) 52–61.
7. G.R. Pettit, M.S. Hoard, D.L. Doubek, J.M. Schmidt, R.K. Pettit, L.P. Tackett, J.C. Chapuis, J. Ethnopharmacol. 53 (1996) 57-63.
8. Jolly ,M.S. (1966) Tasar research scientific Brochure no.4 Central tasar research station, Central Silk Board , Ranchi, pp 144.
9. Jolly ,M.S. Sen S.K. & Maqbool,A. (1974). Tasar culture, Bombay, 266.
10. K. Karthikeyan, B.R.S. Bai, K. Gauthaman, K.S. Sathish, S.N. Devaraj, Cardioprotective effect of the alcoholic extract of *Terminalia arjuna* bark in *in vivo* model of myocardiooc ischemic reperfusion injury. Life Sci. Vol. No.1. P. 73 (2003) 2727–2739.
11. M. Sarwat, M.S. Negi, M. Lakshmikumaran, A.K. Tyagi, S. Das, P.S. Srivastava, Electron. J. Biotechnol. 9 (2006) 86–91
12. M.Sarwat., S. Das., P.S. Srivastava., 2011, AFLP and SAMPL markers for characterization genetic diversity in *Terminalia arjuna* : a backbone tree of Tasar silk industry, Plant Syst Evol, 293:13-23
13. Nei, M. and W. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of National Academy of Sciences (USA), 76: 5269-5273.
14. Rishi A. and Sarin, 2009, R. Estimation of primary metabolites from *Gloriosa superba* L. in vivo and in vitro. Int. J. Mendel.26 (1-4): 87.
15. Rogers, S.O. and Benedich, A.J. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Molecular Biology, 1985, vol. 5, no 2, p. 69-76.
16. Sagwan S., Rao D.V. and Sharma R.A., 2010, Biochemical estimation of primary metabolites from *Pongamia pinnata* (L.): an important biodiesel plant. IJPSRR, 5(1) 146-149.
17. Shan, F.; Clarke, H.C.; Plummer, J.A.; Yan, G. and Siddique, K.H.M. Geographical patterns of genetic variation in the world collections of wild annual *Cicer* characterized by amplified fragment length polymorphisms. Theoretical and Applied Genetics, January 2005, vol. 110, no. 2, p. 381-391.
18. Singh, A.; Negi, M.S.; Rajagopal, J.; Bhatia, S.; Tomar, U.K.; Srivastava, P. S. and Lakshmikumaran, M. Assessment of genetic diversity in *Azadirachta indica* using AFLP markers. Theoretical and Applied Genetics, July 1999, vol. 99, no. 1-2, p. 272-279.
19. Srivastav, P.K., 2003. Tree improvement studies in genus *Terminalia* Linn. Proceedings of National Academy of Sciences, India Sec-B (Bio. Sci.), 73: 95-142.
20. Talreja T., 2011, Biochemical estimation of three primary metabolites from medicinally important plant *Moringa oleifera*, IJPSRR, 7(2): 186-188.
21. V.K. Tripathi, B. Singh, R.K. Tripathi, Upadhyay, V.B. Pandey, Orient. J Chem. I (1996) 1–16.

22. Vishal P. Deshmukh, Prashant V. Thakare, Uddhav S. Chaudhari, Prashant A. Gawande and Vinod S. Undal., (2009) Assessment of Genetic Diversity among Terminalia Species Using RAPD Markers., Global Journal of Biotechnology & Biochemistry 4 (2): 70-74.
23. Yadav A., Sharma R. A., Singh D. and Bhardwaj R., 2012, Biochemical estimation of primary metabolites of *Cassia nodosa* bunch, IJB 3(2): 65-69.

How to cite this article:

Gandhi Nemali *et al.* Identification of elite biotype of terminalia arjuna using rapid molecular marker for production of high yield tasar cocoons. *International Journal of Recent Scientific Research Vol. 6, Issue, 2, pp.2634-2638, February, 2015*
