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Molecular Studies on Some Tree Species Emphasizing DNA

A mini-review

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Abstract: In this review, the information has been provided on the research studies and investigations which were reported earlier by the researchers. The main focus is based on deoxyribonucleic acid (DNA) studies of several tree species. It has been observed that isolation of pure DNA makes the base of those molecular studies which completely rely over the quality of DNA. Hence, several kind of DNA isolation protocols are available in the research world. Many are based on readily available kit methodology, modified cetyltrimethylammonium bromide (cTAB) protocols of genomic DNA isolation, etc. The major objective of these methods is to achieve quality DNA with adequate amount for best outcomes in the molecular studies.

Key word- DNA Isolation, DNA markers, Molecular studies, Tree species

I. INTRODUCTION

Good and high quality of genomic DNA is important for molecular studies. Due to high degrading character of wood, DNA isolation is considered as tough task. In most of the research laboratories DNA isolation kits are available which may provide low DNA yield and these kits are observed as very expensive. Therefore, a DNA isolation procedure was provided, through which DNA can be extracted manually. This standardized DNA isolation method is applicable over extraction of DNA from the explant samples of bark, Sapwood and Heartwood **[1]**. From dried cambium and fresh leaf explant samples of *Mansonia altissima*, high quality of DNA was obtained. In comparison to commonly accepted ratio of DNA purity both fresh leaf and dried cambium has shown low purity **[2]**. An easy method was described to obtain tissue from cambial zone for DNA isolation and testing the relevance of the procedure for several tropical tree species. For obtaining DNA from the tissue samples of eleven species in nine families, the procedure was used with success **[3]**. The quality and concentration of the DNA of *Bombax ceiba* were examined by spectrophotometer **[4]**. By using 100bp DNA ladder as reference the quality and quantity of DNA was examined on 0.8% agarose gel preparation for *Ceiba pentandra* samples **[5]**. Only some of the research studies has shown the capability to extract DNA from dried wood samples such as Sapwood and Heartwood **[1]**. The metagenomic DNA yield from *Quercus brantii* leaf, stem, roots, bulk and rhizospheric soils acquired by One Spin column based method and through indirect SDS based method. These new procedures found efficient **[6]**.

From grafted elite replica of *Quercus robur*, *Prunus avium*, *Fraxinus excelsior*, *Acer pseudopla-tanus* and *Quercus petraea* leaves were collected during the month of October for DNA extraction. To maintain nucleic acids within non-oxidative environment and to denature the endonucleases activity it was observed that 1% β-mercaptoethanol is optimal. At 260 and 280nm absorbance, the DNA concentrations were shown in UV spectrophotometer. The purity of DNA was examined at 260/280nm. The DNA quantification was also confirmed after ethidium bromide staining and observed inside the UV light on the gel which was prepared using 1% agarose in 1X Tris Borate buffer **[7]**. An easy and efficient protocol of DNA extraction from tissue samples of phytoplasma infected woody and herbaceous plants was described for obtaining high quality of DNA for detection of PCR. In this procedure phenol, alcohol or chloroform are not required for nucleic acid precipitation **[8]**. DNA isolation from Heartwood samples is a difficult task due to the presence of chemicals that create problems in DNA amplification. DNA isolation and amplification were successfully done from old aged and degraded Heartwood samples and can be utilized for timber origin studies and investigations **[9]**. The protocols were optimized for isolating highly pure DNA from forty cultivar of Avocado and RNA isolation from different types of tissues. The procedure of DNA isolation provided quality DNA from leaf

tissue samples of forty Avocado cultivar. Further methodology was optimized for RNA isolation from samples of different Avocado plant parts **[10]**. An easy, rapid, cost effective and promising method of DNA isolation was developed for obtaining pure and quality DNA from the recalcitrant plant samples having PCR inhibitors. For developing DNA isolation protocol the acetone efficiency was investigated **[11]**. Latest high throughput sequencing of DNA has provided a "reach" towards number of woody plant genomes beyond the earlier *Eucalyptus* and *Populus* reference genome, references that today consist oak and willow with chestnut and peakon to follow in the coming time **[12]**. For the expression of gene and its sequencing, a protocol which is phenol and chloroform free was developed to isolate nucleic acids from recalcitrant, woody tropical trees tissue samples. DNA was isolated from mature leaves while RNA isolation was done using leaf, root, stem, axillary bud and flowers **[20]**.

II. Random Amplified Polymorphic DNA (RAPD) Analysis

For (RAPD) analysis of *Alstonia scholaris*, a procedure was developed for optimizing DNA isolation and Polymerase chain reaction (PCR) conditions. The outcomes indicated that standardized procedure of DNA isolation and PCR was suitable for plant species of different genera consist of high polysaccharides and polyphenolics and for processing a very large number of samples for genomic analysis and studies **[13]**. A DNA isolation method was developed for tissues of woody plant species. DNA extracted successfully through the implemented procedure from eleven species and five different kind of tissues, was suitable for restriction and RAPD analysis **[14]**.

III. Use of RAPD primers in the studies

The young and older leaves were collected from *Dimorphandra mollis* for DNA isolation. The isolated DNA was examined by amplification through PCR using RAPD primers **[15]**. Amid few cultivated populations of *Terminalia arjuna* low genetic diversity was disclosed by employing DNA fingerprints generated through 10 commercially present RAPD primers i.e., RP101-RP110 **[16]**.

IV. Reports on application of DNA marker

A research investigation related to DNA marker based genetic variation was reported for *Cassia fistula* **[17]**. With respect to *Ficus virens* the phylogenetic tree was constructed by combining morphology and DNA markers. The research investigation supports the importance of employing molecular and morphological data for effective, species discrimination having high level of matching **[18]**.

V. Report on DNA barcoding

DNA barcoding broadly implemented to recognize the composition of plant species in respected tropical and temperate ecosystems. Very few studies have considered DNA barcodes for the documentation of woody and herbaceous constituents of forest plot. Therefore, a study was reported and represented as one of the first applications and utilization of DNA barcodes in Southwest China's subalpine forest dynamics plot **[19]**. By the researchers of this research study, the information regarding DNA barcoding was very well provided.

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VI. CONCLUSION

Reports on the research studies provided in this review focusing on woody plants and trees may further bestow researchers with vast amount of in-depth information and understanding, during future research investigations. The advancement in DNA studies may provide path for several molecular applications with respect to woody plants.

VII. CONFLICT OF INTEREST: Declared none

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REFERENCES

[1] Fatima, T., Srivastava, A., Hanur, V.S. & Srinivasa Rao, M. (2018). An effective wood DNA extraction protocol for three economic important timber species of India. *American Journal of Plant Sciences*, 9, 139-149. https://doi.org/10.4236/ajps.2018.92012

[2] Lawal, A.& Agbator, V.E. (2021). Extraction of high-quality genomic DNA from *Mansonia altissima* for microsatellite analysis. *Journal of Research in Forestry, Wildlife & Environment*, 13(4), 115-119.

[3] Colpaert, N., Cavers, S., Bandou, E., Caron, H., Gheysen, G., & Lowe, A.J. (2005). Sampling tissue for DNA analysis of trees: Trunk cambium as an alternative to canopy leaves. *Silvae Genetica*, 54 (6), 265-269. doi:10.1515/sg-2005-0038.

[4] Ju, M.M., Fu, Y., Zhao, G.F., He, C.Z., Li, Z.H. & Tian, B. (2018). Effects of the Tanaka Line on the genetic structure of *Bombax ceiba* (Malvaceae) in dry-hot valley areas of southwest China. *Ecology and Evolution*, 8, 3599-3608. doi: 10.1002/ece3.3888

[5] Abengmeneng, C.S., Ofori, D., Kumapley, P., Akromah, R., Jamnadass, R. & Quain, M. (2016). Genetic relationship among 36 genotypes of *Ceiba pentandra* (L.) as revealed by RAPD and ISSR markers. *American Journal of Agriculture and Forestry*, 4(4), 86-96. doi: 10.11648/j.ajaf.20160404.13.

[6] Ahmadi, E., Kowsari, M., Azadfar, D. & Jouzani, G.S. (2018). Rapid and economical protocols for genomic and metagenomic DNA extraction from oak (*Quercus brantii* Lindl.). *Annals of Forest Science*, 75(43), 1-14. https://doi.org/10.1007/s13595-018-0705-y

[7] Lefort, F. & Douglas, G.C. (1999). An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species *Acer, Fraxinus, Prunus and Quercus*. *Annals of Forest Science*, 56(3), 259-263. https://doi.org/10.1051/forest:19990308

[8] Green, M.J. & Thompson, D.A. (1999). Easy and Efficient DNA Extraction from Woody Plants for the Detection of Phytoplasmas by Polymerase Chain Reaction. *Plant Disease*, 83 (5), 482-485.

[9] Akhmetzyanov, L., Copini, P., Sass-Klaassen, U., Schroeder, H., de Groot, G.A., Laros, I. & Daly, A. (2020). DNA of centuries-old timber can reveal its origin. *Scientific Reports*, 23;10(1), 20316. doi: 10.1038/s41598-020-77387-2.

[10] Nath, O., Fletcher, S.J., Hayward, A., Shaw, L.M., Agarwal, R., Furtado, A., Henry, R.J. & Mitter, N. (2022). A Comprehensive High-Quality DNA and RNA Extraction Protocol for a Range of Cultivars and Tissue Types of the Woody Crop Avocado. *Plants*, 11, 242. https://doi.org/ 10.3390/plants11030242

[11] Gouker, F. E., Guo, Y. & Pooler, M.R. (2020). Using acetone for rapid PCR-amplifiable DNA extraction from recalcitrant woody plant taxa. *Applications in Plant Sciences*, 8(12): e11403. doi:10.1002/aps3.11403

[12] Tuskan, G.A., Groover, A.T., Schmutz, J., DiFazio, S.P., Myburg, A., Grattapaglia, D., Smart, L.B., Yin, T., Aury, J-M., Kremer, A., Leroy, T., Le Provost, G., Plomion, C., Carlson, J.E., Randall, J., Westbrook, J., Grimwood, J., Muchero, W., Jacobson, D. & Michener, J.K. (2018) Hardwood Tree Genomics: Unlocking Woody Plant Biology. *Frontiers in Plant Science*. 9:1799. doi: 10.3389/fpls.2018.01799

[13] Sreedevi, E. & Pullaiah, T. (2019). DNA Extraction and Assessment of genetic variability through RAPD analysis for Molecular Studies in *Alstonia scholaris* (L.) R. Br. *Journal of Emerging Technologies and Innovative Research*, 6(6), 480-487. http://www.jetir.org/papers/JETIR1908229.pdf

[14] Cheng, F.S., Brown, S.K. & Weeden, N.F. (1997). A DNA extraction protocol from various tissues in woody species. *HortScience*, 32(5), 921-922.

[15] Moreira, P.A. & Oliveira, D.A. (2011). Leaf age affects the quality of DNA extracted from *Dimorphandra mollis* (Fabaceae), a tropical tree species from the Cerrado region of Brazil. *Genetics and Molecular* Research, 10(1), 353-358. doi: 10.4238/vol10-1gmr1030.

[16] Sairkar, P.K., Chouhan, S., Sen, A., Sharma, R. & Singh, R.K. (2017). Genetic drift in six cultivated populations of *Terminalia arjuna*. *Journal of Applied Biology & Biotechnology*, 5 (4), 48-56. doi: 10.7324/JABB.2017.50408

[17] Kumar, A., Tripathi, V. & Pushpangadan P. (2007). DNA marker-based genetic variation in *Cassia fistula* L. *Indian Journal of Genetics and Plant Breeding*, 67(2), 173-176.

[18] Mahima, K., Sudhakar, J.V. & Sathishkumar, R. (2020). Molecular phylogeny of the *Ficus virens* complex (Moraceae). Genome, 63(12), 597-606. https://doi.org/10.1139/gen-2019-0210

[19] Tan, S-L, Luo, Y-H, Hollingsworth, P.M., Burgess, K.S., Xu, K., Li, D-Z. & Gao, L-M. (2018). DNA barcoding herbaceous and woody plant species at a subalpine forest dynamics plot in Southwest China. *Ecology and Evolution*, 8:7195–7205. https://doi.org/10.1002/ece3.4254

[20] Barbier, F.F., Chabikwa, T.G., Ahsan, M.U., Cook, S.E., Powell, R., Tanurdzic, M. & Beveridge, C.A. (2019). A phenol/chloroform-free method to extract nucleic acids from recalcitrant, woody tropical species for gene expression and sequencing. *Plant Methods* 15, 62. https://doi.org/10.1186/s13007-019-0447-3