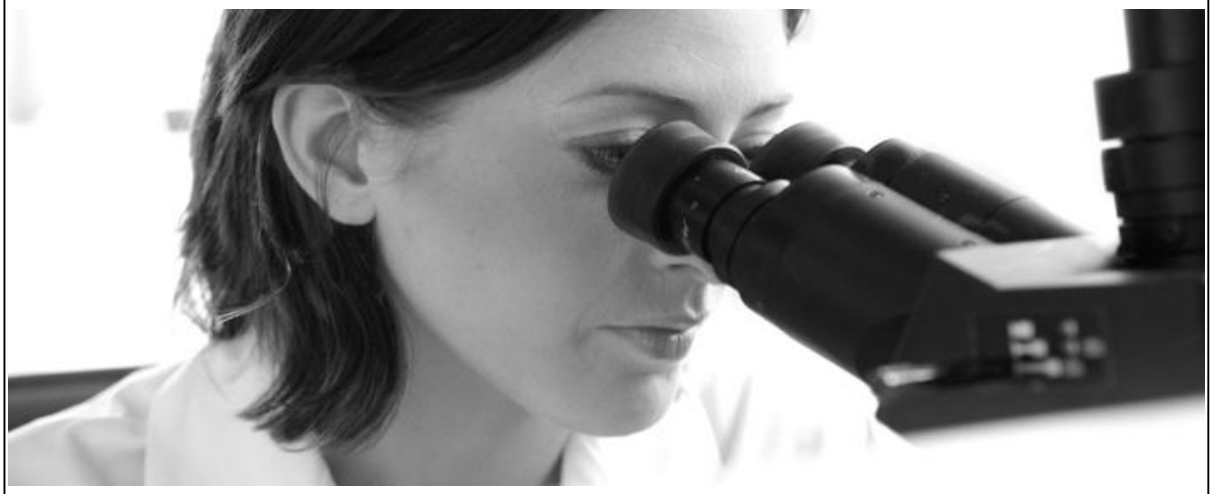
ISSN: 0976-3031

International Journal of Recent Scientific Research

Impact factor: 5.114

GENETIC RELATIONSHIP OF SOME ECONOMICALLY IMPORTANT MEDICINAL PLANTS OF NORTHEAST INDIA



Tunginba SinghY., Vanlalsanga and Supriya Devi N

Volume: 6 Issue: 10

THE PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH
http://www.recentscientific.com
E-mail: recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 10, pp. 7118-7120, October, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

GENETIC RELATIONSHIP OF SOME ECONOMICALLY IMPORTANT MEDICINAL PLANTS OF NORTHEAST INDIA

Tunginba Singh Y*., Vanlalsanga and Supriya Devi N

Department of Botany, Mizoram University, Aizawl, Mizoram, India

ARTICLE INFO

Article History:

Received 05th July, 2015
Received in revised form
08th August, 2015
Accepted 10th September, 2015
Published online 28st
October, 2015

Key words:

Medicinal plants, NE India,
RAPD, polymorphism, genetic
diversity

ABSTRACT

Six economically important medicinal plants which are also used as food by locals of Northeast India were analysed for genetic relatedness and diversity. RAPD markers were used in the present investigation where a total of 181 polymorphic bands were amplified with 100% polymorphism. A UPGMA tree constructed using Nei's genetic distance measure showed three clears clusters. This study will provide a preliminary data for conservation of economically important medicinal plants of NE India.

INTRODUCTION

Since time immemorial man had been using plants as medicine for curing several kinds of diseases like asthma, bronchitis, diabetes, skin disorders, dysentery, stomach ulcer etc. The different compositions of the complex chemical substances present in medicinal plants make them a curative property, which occurs as secondary plant metabolites in one or more parts and according to their composition, are grouped as alkaloids, glycosides, corticosteroids and essential oils and so on (Raja and Sreenivasulu, 2015; Chikkaswamy, 2015). WHO reported that about 80% of the world's population rely on medicinal plants for their healthcare (WHO, 1993). However, these useful biotic resources have become threatened due to over exploitation by humans (Singh and Singh, 2009). A large number of medicinal plants are facing serious genetic loss and extinction while their detail information is still lacking (Nag *et al*., 2014).

Many populations of medicinal plants accumulate pharmaceutical properties of differential quality; and knowledge about their variability and quality is necessary for thoughtful use, conservation, and protection of genetic diversity (Dusek *et al*., 2010). Thus, understanding the genetic variation within populations is necessary to establish proper conservation and preservation of these precious resources (Mulligan and Findley, 1970; Bansal *et al*., 2012). Genetic diversity is also important for understanding evolutionary history of a species and for its future risk management. For this matter, molecular markers are successfully used to measure genetic diversity and relationships. RAPD markers are neutral, arbitrary, highly reproducible, cheap, fast and easy to use molecular markers, which can examine genetic variations without knowing prior information of the genome (Williams *et al*., 1990; Hadrys *et al*., 1992). RAPD markers have been useful for assessing genetic diversity of various medicinal plants (Lal *et al*., 2011; Gahlaut *et al*., 2013; Pourmohamad, 2013; Sairkar *et al*., 2013). The NE states of India, comprising of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura, lie within the international boundaries of Bhutan and China in the north, Bangladesh in the south eastern and Myanmar in the west.

The region lies in the Indo-Burma biodiversity hotspot (Myers 1988). This region is inhabited by various ethnic groups of people who speak different dialects and perform different cultural practices. It has rich reserve for medicinal plants providing potential abilities of Ayurvedic, Siddha and Unani medicines. In this study, six economically important medicinal plants, namely *Centella asiatica*, *Phlogacanthus thyrsiflorus*, *Zanthoxylum rhetsa*, *Emilia sonchifolia*, *Cissus adnata* and *Clerodendrum colebrookianum* were used to investigate genetic relationship and variation employing RAPD markers.

Corresponding author:* **Tunginba SinghY
Department of Botany, Mizoram University, Aizawl, Mizoram, India

These plants are also consumed as vegetables by local people of North Eastern (NE) region of India. Also most of them are commonly found in local markets.

MATERIALS AND METHOD

Plant sample collection and DNA Isolation

Fresh young leaves of *Centella asiatica*, *Aralia armata*, *Emilia sonchifolia, Phlogacanthus thyrsiflorus*, *Cissus adnata* and *Clerodendrum colebrookianum,* growing in and around Mizoram University Campus, Aizawl, Mizoram, India, were collected for the study. The plants specimens were identified at Department of Botany, Mizoram University. Economic importance and medicinal properties of these plants are listed in Table 1. Genomic DNA was isolated from the above plants using Hipure-plant-DNA Isolation kit (Himedia, India) following manufacturer's instruction.

PCR Amplification

PCR amplification was performed in 10 µl reaction volumes containing 10ng template, 30 ng/µl of each primer, 200 µM of each dNTP (Himidea, India) 0.5 U Taq (Promega, USA) in a Veriti 96 Well thermal cycler (ABI, USA) with a profile of 35 cycles of denaturation at 95^0C for 30 seconds, primer annealing at 37^0C for 30 seconds and primer extension at 72^0C for 1 minute followed by final extension for 7 minutes. PCR products were electrophoresed on 1.5 % agarose gel, visualised by ethidium bromide staining and photographed under UV using Alpha Imager (Protein Simple, USA).

Genetic analyses

Amplified products obtained from RAPD-PCR were scored for the presence (1) and absence (0) of the band. Only reproducible bands in the size range of 100 bp to 2 kb were scored. Sizes of the bands were ascertained using Alpha View SA ver 3.4.0.0 (Protein Simple, USA) with reference to DNA size marker.

The data was then used to estimate percentage polymorphism, genetic diversity (Nei 1972), genetic distance and UPGMA tree construction using POPGEN ver 1.32 (Yeh *et al* 1999). Phylogenetic tree was drawn using a freeware Tree View.

RESULT AND DISCUSSION

RAPD markers are proved to be useful and inexpensive system for assessing genetic diversity and relatedness. Though this marker is dominant in nature yet they are efficient in providing useful molecular data in lesser known genomes and also in taxonomic identifications (Hadrys *et al* 1992).

A total of 10 random RAPD primers were employed to screen genetic diversity and relatedness of economically important medicinal plants, out of which 8 primers were polymorphic (Table 2). These markers detected 181 reproducible bands giving 100% polymorphism. The polymorphism was able to classify the six studied plants. Primer OPA01 (Figure 1) produced the maximum number of bands (29) while minimum bands were amplified with OPB01 (19).

Table 2 shows numbers of bands amplified by individual primers. The highest genetic distance was observed between *Phlogacanthus thyrsifloris* and *Emilia sonchifolia* (0.5834) while least diversity was found between *Emilia sonchifolius* and *Cissus adnata* (0.3863).

Gene diversity for all loci was found to be moderate at 0.3201 (±0.0769) suggesting the need for designing conservation strategies for the studied medicinal plants. A UPGMA tree constructed using Nei's genetic distance measure showed three clusters: Cluster I comprising *Centella asiatica*, *Zanthoxylum rhetsa*; Cluster II comprising *Emilia sonchifoli*, *Cissus adnata* and *Clerodendrum colebrookianum* while Cluster III was represented by *Phlogancanthus thyrsifolus* (Figure 2).

A 1.5% Agarose Gel

Table 1 Economic importance and medicinal use of the studied plants

S No.	Medicinal Plant	Family	Local Name*	Medicinal use
1	*Centella asiatica* (L.) Urban.	Apiaceae	Peruk (MN), Lambak (MZ)	Skin deseases, dysentery, urinary, stomach ulcer.
2	*Phlogacanthus thyrsiflorus* Nees.	Acanthaceae	Nongmangkha (MN)	Expectorant in cough, asthma, fever
3	*Zanthoxylum rhetsa* (Roxb.) DC.	Rutaceae	Ching-it (MZ)	Asthma, stomach problem
4	*Emilia sonchifolia* (L.) DC.	Asteraceae	Tera Paibi Macha (MN)	Bowel complaints and leaf juice is prescribed in sore eyes and night blindness
5	*Cissus adnata* Roxb.	Vitaceae	Kongouyen (MN)	Urinary troubles due to stone, powdered roots are use as antiseptic in cuts and wounds.
6	*Clerodendrum colebrookianum* Walp.	Verbenaceae	Kuthap (MN), Phuihnam (MZ), Nephaphu (AS), Umrem (NA)	Rheumatic pains, blood pressure

*MN= Manipuri, MZ= Mizo, AS= Assamese, NA=Nagamese

Table 2 RAPD primers used in the present study

Primers	Sequences	No. of bands amplified
OPA-01	5'-CAGGCCCTTC	29
OPB-01	5'-GTTTCGCTCC	19
OPA-02	5'-TGCCGAGCTG	20
OPA-03	5'- AGTCAGCCAC	22
OPA-04	5'- AATCGGGCTG	24
OPB-04	5'- GGACTGGAGT	22
OPA-05	5'- AGGGGTCTTG	20
OPB-05	5'- TGCGCCCTTC	25

Medicinal plants have been utilized for their curative properties due to easy access, low cost and ancestral experience (Marini-Bettlo 1980). But the pharmaceutical uses of these important medicinal plants are strictly connected with the content of their genetic potential and the climatic conditions (Dusek *et al* 2010). Genetic diversity measures will be useful in understanding genetic potentials of a plant. To the best of our knowledge, no study on genetic diversity of economically important medicinal plants of Northeast India has been done thus far at molecular level.

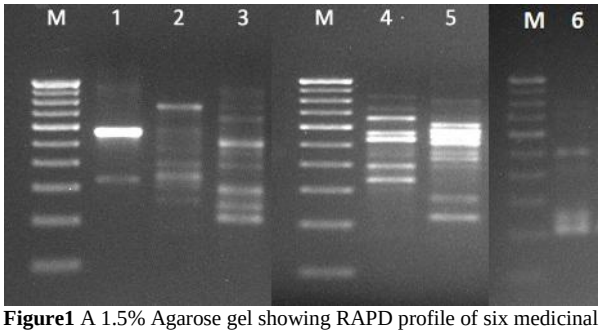


Figure1 A 1.5% Agarose gel showing RAPD profile of six medicinal plants with OPA01. (M=100bp size marker, 1= *Centella Asiatica*, 2= *Zanthoxylum rhetsa*, 3= *Phlogacanthus thyrsiflorus*, 4= *Emilia sonchifolius*, *5= Clerodendrum colebrookianum* and 6= *Cissus adnata*)

Phylogenetic tree

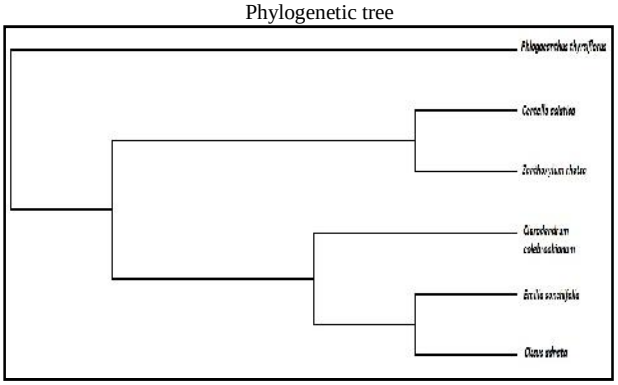


Figure 2 UPGMA tree showing clustering of studied medicinal plants using RAPD markers

Hence, an attempt was made to investigate genetic relatedness and variation among six economically important medicinal plants, namely *Centella asiatica*, *Phlogacanthus thyrsiflorus*, *Zanthoxylum rhetsa*, *Emilia sonchifolius*, *Cissus adnata* and *Clerodendrum colebrookianum* using RAPD markers. The markers were able to assess genetic diversity and relationship among studied economically important medicinal plants.

CONCLUSION

Six economically important medicinal plants of Northeast India were successfully assessed for genetic diversity and relatedness. The finding will provide preliminary data for designing conversation strategy of medicinal plants in the region. More plants need to be investigated in order to understand in depth knowledge about relationship of various medicinal plants.

Reference

1. Bansal, D., Bhasin, P., Yadav, O.P., and Punia, A. 2012. Assessment of genetic diversity in Lepidium sativum (Chandrasur) a medicinal herb used in folklore remedies in India using RAPD. *Journal of Genetic Engineering and Biotechnology*, 10, 39–45.
2. Chikkaswamy B.K. 2015. Assessment of genetic diversity and relationships of medicinal plants using RAPD marker. *International Journal of Advanced Research in IT and Engineering*, 4 (2), 25-35.
3. Dušek, K. Dušková, E., and Sméka lová K. 2010. Genetic Diversity of Selected Medicinal Plants in Protected Landscape Areas in the Czech Republic. *Czech Journal of Genetics and Plant Breeding*, 46:34–36.
4. Gahlaut A., Gothwal A., Hooda V., and Dabur R. 2013. RAPD patterns of some important medicinal plants and their substitutes used in Ayurveda to identify the genetic variations. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5 (1), 239-241.
5. Hadrys H., Balick M., and Schierwater B., 1992. Application of random amplified polymorphic DNA (RAPD) in molecular ecology, Molecular Ecology, 1, 55-63.
6. Lal S., Mistry K.N., Vaidya P.B., Shah S.D., and Thaker R.A., 2011. Genetic diversity among five economically important species of *Asparagus* collected from central Gujarat (India) utilizing RAPD markers (Random Amplification of Polymorphic DNA). *International Journal of Advanced Biotechnology and Research*, 2 (4), 414-421.
7. Marini-Bettolo G.B., 1980. Present aspects of the use of Medicinal plants in traditional medicine. *Journal of Ethnopharmacology*, 2, 5–7.
8. Myers, N. 1988. Threatened biotas: `hotspots' in tropical forests. Environmentalist 8, 187-208.
9. Mulligan, G.A., and Findlay, J.N., 1970. Sexual reproduction and agamospermy in the genus Draba. *Canadian Journal of Botany*, 48, 269-270. doi:10.1139/b70-040
10. Nag A., Ahuja, P.S., and Sharma R.K., 2014. Genetic diversity of high-elevation populations of an endangered medicinal plant. AOB Plants, 7: plu076; doi:10.1093/aobpla/plu076.
11. Nei M., 1972. Genetic distance between populations. American Naturalist, 106, 283-92.
12. Paramanik, R.C., and Chikkaswamy B.K., 2014. Study on Genetic Diversity relationship some Medicinal plants using RAPD Molecular marker *International Journal of Current Microbiology and Applied Sciences*, 3(6): 1004-1015.
13. Pourmohammad, A,. 2013. Application of molecular markers in medicinal plant studies. Acta Universitatis Sapientiae Agriculture and Environment, 5, 80-90.
14. Raja, R., and Sreenivasulu, R.M., 2015. Medicinal plants secondary metabolites used in pharmaceutical importance-an overview. *World Journal of Pharmacy and Pharmaceutical sciences*. 4(4):436-447.
15. Sairlar, P., Vijay, N., Batav, N., Silawat, N., Garg, R.K., Sharma, R., Chouhan, S., Mehrotra, N.N. 2013. Genetic variability in two economically important medicinal plants of Madhya Pradesh, India *Withania somnifera* and *Rauwolfia serpentina* using RAPD markers. Asian Journal of Experimental Biological Sciences, 4 (1), 36-43.
16. Singh, R., and Singh, M., 2009. Wild medicinal plants of Manipur include in the red list. Asian Agri-History, 13 (3), 221-225.
17. Yeh, F.C.,Yang, R., and Boyle, T., 1999. POPGENE ver 1.32 Microsoft window-based freeware for population genetic analysis (University of Alberta, Canada).
18. WHO., 1993. Guidelines on the conservation of medicinal plants. Switzerland: IUCN, WHO & WWF.
19. Williams, J.G.K., Kubelik, A.R., Kenneth, J.L., Rafalski, J.A., and Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research, 18 (22), 6531-6535.

International Journal of Recent Scientific Research

ISSN 0976-3031