International Journal of Advanced Research in IT and Engineering

ISSN: 2278-6244
Impact Factor: 4.054

ASSESSMENT OF GENETIC DIVERSITY AND RELATIONSHIPS OF MEDICINAL PLANTS USING RAPD MARKER

B.K.Chikkaswamy*

Abstract: *Molecular genetic fingerprints of medicinal species were developed using Randomly Amplified Polymorphic DNA (RAPD) marker to elucidate the genetic diversity among the 18 species . DNA was isolated using the CTAB method. The amplification was accomplished by using 10 primers and the specific PCR working program. Three decamer-primers generated 250 RAPD fragments, of which 232 fragments were polymorphic with 96.84% of polymorphism. Some of the RAPD markers were useful for species discrimination and identification. Most of the RAPD markers studied showed different level of genetic polymorphism. Amplified fragment sizes ranged from 300 to 5000 bp. Pairwise Nei and Li's similarity coefficient value ranged from 0.00 to 0.72 for 18 species of medicinal plants . A dendrogram was constructed based on the unweighted pair group method using arithmetic averages. Cluster analysis of data using the UPGMA algorithm placed the 18 species of medicinal plants into four groups that are somewhat congruent with classification based on morphological characters proposed by earlier works. This analysis grouped all species into different clusters and clearly differentiated of medicinal plants into separate groups. This method of analysis can be helpful in selecting diverse parents and give broadness to the germplasm base of medicinal plants breeding programs in the future.*

Key Words: *Medicinal plants, RAPD, Genetic Diversity*

*Sigma BioScience Research Center, Indira Nagar, Bangalore

1. INTRODUCTION

The world is endowed with a rich wealth of medicinal plants. Herbs have been the principal form of medicine in ancient India. White herbs lost their importance due to pharmaceutics-d revolution. They are also becoming popular as people strive to live healthy in the face of chronic stress and pollution and to treat illness with medicines that work in concert with body's relied defense. Medicinal plants play a crucial role in the lives of rural people, in remote parts of developing countries with limited facilities for health care (Purohit and Prajapati, 2003).

Around 70,000 plant species, from lichens to flowering trees have been used for medicinal purposes. Many species are used in herbal medicines and is used in unrefined or semi-processed form, often as mixtures, which may also contain non-botanical ingredient. A few species are the sources of defined compounds used in the pharmaceutical industry. There is international trade in medicinal plants used in herbal medicine and in the manufacture of pharmaceuticals. There is also a growing interest in obtaining samples of plant material and traditional knowledge about the uses of plants and also to explore commercial medicinal products. The scale of international trade in medicinal plants is difficult to assess, because of the paucity of reliable statistics and trade secrecy. Ancient Indian literature incorporates a broad definition of medicinal plants and considers "all" plant entities to be potential sources of medicinal substances. While all plant entities are potentially medicinal, only those plants are considered 'medicinal' whose medicinal use has already been discovered for human or animal application. Such an application can neither be in the western biomedical system nor in homeopathy or any of the traditional systems of medicine like Ayurveda, Unard, Siddha and Swa-Rigpa, and folk medical traditions, which are eco-system and ethnic community specific (Shankar and Veda, 2003).Traditionally medicinal plants have been used for human, veterinary and plant health. There are medical texts that deal with the treatment of cows; horses, elephants, and birds, there are also texts on subjects like Vriksh Ayurveda and Krishi Sastra that deal with the use of plants for controlling pests, treating plant diseases and as biofertilizers.

Conservation of Medicinal Plants

A taxon is Endangered when it is not critically endangered but is facing a very high risk of extinction in the wild in the near future (Ravikumar and Ved, 2000).RAPD, markers behave

as dominant genetic markers, meaning that in a segregating population the homozygote of the parental type from which a given RAPD, ISSR and SSR band is amplified cannot be distinguished from the heterozygote, because the heterozygote also produces a RAPD, band.The only unambiguously assigned a genotype is the homozygote of the other parental type (no RAPD band). The segregating F2 population may therefore be scored as follows band present: AA or Ab; band absent: bb. Remembering this fact, it is easy to select the populations best suited for the construction of genetic maps with RAPD markers:

2. MATERIALS AND METHODS

The materials required and methodology of the present work is carried out at Department of Biotechnology, Acharya Institute of Technology, Karnataka and Plant molecular biology laboratory, Department of Horticulture, Hulimavu Biotechnology Centre, Govt of Karnataka, Bagalore, India. In the year 2010-2012.The materials used and methods followed in the study are presented here. Fresh, young, disease free leaves of 18 medicinal plants *Hemigraphis colorata, Marjorana hortensis, Artemisia vulgaris, Artemisia pallens, Ocimum sanctum, Ocimum basilicum, Ocimum hratissimum, Mentha piparita, Mentha citrate, Mentha spicata, Acorus calamus, Centella asiatica, Bacopa moninierii, Piper longum, Piper nigrum, Clitoria ternatea, Aloe vera, Stevia rebaudiana,* which were collected from the germplasm maintained at the different regions of Karnataka as medicinal plants germplasm conservation.

DNA Extraction

Fresh, young and disease free leaves of 6 different medicinal plants were collected and immediately kept in ice to reduce the nuclease activity. It was brought to the laboratory, weighed (2 gms each), and frozen in liquid nitrogen and stored at -70ºC till further use. The DNA was extracted using the CTAB method (Porebski *et. al*., 1997) with certain modifications. 2gms of fresh medicinal plant leaf material was ground into a fine powder using liquid nitrogen. The powder was then transferred to sterile centrifuge tubes and 12ml of extraction buffer was added, mixed thoroughly and incubated 65ºC in a water bath for one hour with intermittent shaking. The tubes were brought to room temperature and centrifuged at 8000 rpm for 10 min at 4ºC. The supernatant was transferred to new tubes, 6 ml of chloroform: isoamyl alcohol (24:1) was added and mixed thoroughly. The tubes were centrifuged at 8000 rpm for 10min at 4ºC. The supernatant was transferred to new tubes

and repeated the same steps twice. The DNA was then precipitated by adding half volume of 5M NaCl, an equal volume of chilled propanol and incubated at 4ºC over night. DNA was pelleted by centrifuging at 20,000 rpm for 12 min at 4ºC. The pellets were dried after adding 70% ethanol and 1ml of TE buffer was added to which 20 µl of RNase was added. This was incubated at 37ºC for one hour and added 300 µl of saturated phenol. It was mixed, centrifuged at 8000rpm for 10 min at 4ºC. The supernatant was transferred to another tube and repeated the same process by adding phenol: chloroform and chloroform respectively. The supernatant was treated with equal volume of isopropanol and incubated at 4ºC for overnight. The DNA was pelleted by centrifuging at 12000 rpm for 20min. The pellet was washed with 70% ethanol and dried. Around 300 µl of TE buffer was added to dissolve the pellet and stored at -20ºC for further use.

Data Analysis

DNA binding patterns generated by RAPD, were scored as ' 1 ' for the presence of band and '0' for its absence. All RAPD assays were performed twice and only the reproducible bands were scored. A similarity matrix was generated using a dendrogram was constructed based on distance matrix data sets by applying Ward's method for cluster analysis using 'STATISTICA' 5.0 computer program.

Table.1. Sequence information on RAPD oligonculeotide primers used for amplification and polymorphism study in 18 medicinal plants.

S.No	RAPD Primers	Sequence (5'-3")
1	OPC - 7	GTCCCFACGA
2	OPL -11	ACGATGAGCC
3	OPO - 08	GCTCCAGTGT
4	OPAH - 15	CTACAGCGAG
5	OPAM - 20	ACCAACCAGG
6	OPAN - 01	ACTCCAGGTC
7	OPAO - 01	AAGACGACGG
8	OPAP - 20	CCCGGATACA
9	OPAN - 05	GGGTGCAGTT
10	OPAP - 10	TGGGTGATCC
11	OPAA - 01	AGACGGCTCC
12	OPAB - 01	CCGTCGGTAG
13	OPAB - 05	CCCGAAGCGA
14	OPAB - 14	AAGTGCGACC
15	OPAH - 13	TGAGTCCGCA
16	OPAF - 02	CAGCCGAGAA
17	OPAJ- 19	ACAGTGGCC
18	OPX - 20	CCCAGCTAGA
19	OPA - 08	GTGACGTAGG
20	OPD - 13	GGGGTGACGA

Table 2. Showing the thermal profile used for RAPD

Temperature	Time	Steps
93ºC	2min	Initial denaturation
45 cycles of 93ºC	1 min	Denaturation
35ºC	1 min	Annealing
72ºC	2 min	Extension
72ºC	7min	Final extension
4ºC	∞	Hold

3. RESULTS

the genomic dna of 18 medicinal plants viz , *hemigraphis colorata, marjorana hortensis, artemisia vulgaris, artemisia pallens, ocimum sanctum, ocimum basilicum, ocimum hratissimum, mentha piparita, mentha citrate, mentha spicata, acorus calamus, centella asiatica, bacopa moninierii, piper longum, piper nigrum, clitoria ternatea, aloe vera, stevia rebaudia* were amplified with oligonucleotides primers

RAPD analysis of medicinal plants using Primer OPAB– 05

The genomic DNA of 18 medicinal plants was amplified with decamer oligonucleotide primers such as **OPAB-05** and as shown in **Fig 1.** The distinct and abundant RAPD fragments were recorded. The total numbers of bands were **generated 51 RAPD gel** profiles. The sizes of the RAPD bands were placed in between 300 – 5000bp in length. The primer produced distinct polymorphic banding pattern in all the plant medicinal plant species, the number of RAPD bands per primer were 2.8 as expected to sexually reproduce plants. The RAPD bands distributed in the plant is important to know the value of breeding patterns in medicinal plants. The number of RAPD bands was produced to reveal Mendelian inherited character, and number scoring revealed medicinal characters. The banding patterns are important and distinct in medicinal plants. The polymorphism was very high and RAPD values were useful to distinguish between the medicinal plant species, apparently diverse elements such as diploid and other exotic species character. The identification of RAPD is very unique in plants, because medicinal value and coupled with highly cross – pollinated and revealed heterozygous character. In the present data the plants like 1 showed one band and remain was revealed 7 to 3 RAPD bands respectively. Further, 1 and 2 medicinal plants showed 1 and 3 RAPD bands due to amplification of primer with the genomic DNA of these plant species. However, it was observed that some of the plants viz., 11 to 12 were recorded 7

bands indicating diverse character compared to other plants. Polymorphic distribution as far as gene flow is concerned revealed high or low speciation. This has been used for various other calculations of medicinal plant breeding programs. Therefore, amplification of genomic DNA of these medicinal plants revealed moderate diversity among them.

RAPD analysis of 18 medicinal plants using Primer OPAB – 14

The data obtained in the present investigation revealed a total number **of 111 RAPD** bands. The genomic DNA of 18 medicinal plants amplified with **OPAB –14** revealed both monomorphic and polymorphic RAPD bands. The distinct and abundant RAPD fragments were recorded. The total number of bands generated lie in between 300 – 5000bp in length. The primer produced medium low and high resolution of RAPD bands. The number of bands per primer was recorded maximum of 6.1 bands. However four bands were recorded in plants like 1 to 18 respectively. Despite, the plants revealed a total **number of 111** bands, therefore the distribution of banding patterns are common, and one of the plants as revealed six RAPD bands due to the amplification of genomic DNA with primer **OPAB –14 .** However, amplification showed very clear and distinct bands, and some of the medicinal plants like 1 to 18 have revealed four bands respectively. From this data it is possible to identify species specific band for medicinal plants for selection, in turn it helps for the cultivation of medicinal plants. The RAPD banding techniques is useful for selection multiplication and introgression of certain traits for breeding of medicinal plants as shown in Fig.2.

RAPD analysis of 18 medicinal plants using Primer OPAH -13

The data obtained in the present investigation revealed a total number **of 88 RAPD** bands. The genomic DNA of 18 medicinal plants amplified by **OPAH -13** revealed both monomorphic and polymorphic RAPD bands. The distinct and abundant RAPD fragments were recorded. The total number of bands generated lie in between 300 – 5000bp in length. The primer produced medium low and high resolution of RAPD bands. The number of bands per primer was recorded maximum of 4.8 bands. However, four bands were recorded in plants like 1 to 18 respectively. Despite, the plants revealed a total **number of 88** bands, therefore the distribution of banding patterns is common, and one of the plants as revealed five RAPD bands due to the amplification of genomic DNA with primer **OPAH -13.** However, amplification showed very clear and distinct bands, and some of the medicinal plants from 1

to 8 have revealed 8 bands respectively. From this data it is possible to identify species specific band for medicinal plants for selection, in turn it helps for the cultivation of medicinal plants. The RAPD banding techniques are useful for selection multiplication and introgression of certain traits for breeding of medicinal plants as shown in Fig 3.

4. DISCUSSION

Genetic resources available for medicinal plant improvement are abundant within plant species. Even though a few species of medicinal plants occur naturally in India, many cultivated medicinal plant species do find their origin within the country especially India. Almost all the cultivated and naturally occurring medicinal plants and which are classified under different family and species, cross pollinate with each other and produce fertile offspring showing no signs of sexual incompatibility characteristic of medicinal plant species. This fact suggests a close genetic or non genetic relationship among the medicinal plants. The present study involving 18 medicinal plants with molecular characterization, of RAPD, analysis for further supports this view.

RAPD analysis of medicinal plants using Primer OPAB– 05 The results of the present investigation on genomic DNA of 18 medicinal plants viz , *Hemigraphis colorata, Marjorana hortensis, Artemisia vulgaris, Artemisia pallens, Ocimum sanctum, Ocimum basilicum, Ocimum hratissimum, Mentha piparita, Mentha citrate, Mentha spicata, Acorus calamus, Centella asiatica, Bacopa moninierii, Piper longum, Piper nigrum, Clitoria ternatea, Aloe vera, Stevia rebaudia* were amplified with oligonucleotides primers **OPAB– 05 revealed total of 51 RAPD bands**, (Fig 1- 4). Similar observations were recorded by Girish Naik and Dandin 2006, Souframani and Gopalakrishna, 2004. Similar observations have also made in other species at cultivars level (Colombo et al., 1998, Banerjee et al., 1999, Das et al., 1998,). **RAPD analysis of 18 medicinal plants using Primer OPAB – 14** were amplified with OPD-07 revealed a **total of 111 RAPD bands.** With an average **of 6.1 bands per** primer, all the 18 medicinal plants exhibited 10 RAPD bands respectively. Whereas the some of the medicinal plants such as *Artemisia vulgaris Mentha citrate and Piper longum* have expressed more than 10 RAPD bands per primer. as shown in **Fig 2-5.** Further, similar observation was made by Awasthi et al., (2004) Basha, S.D and Sujatha, M. 2007 in mulberry and medicinal plants. **RAPD analysis of 18 medicinal plants using Primer OPAH -13 .** It is known from the literature that the molecular markers in medicinal plants is important observations (Girish

Naik and Dandin 2002,). The genomic DNA of 18 medicinal plants was amplified with the primer sequence of OPL –11 produced 3.5 bands per primer. A total of 63 RAPD band discrete amplified products were generated with the primer OPL – 11. Out of these 63 RAPD bands of the medicinal plants, the medicinal plant such as *Acorus calamu* is revealed 10 RAPD bands. OPL – 11 have revealed 7-8 RAPD bands respectively as shown in fig 3-16. **The Primer OPO – O8.** Revealed a **total of 88 RAPD bands**. **Out of these 88**RAPD **bands** indicating both monomorphic and polymorphic characters in the medicinal plants like 1, 7, 8, 13, 15 and 18, significance of cross pollination is slow in these plants and out crossing is less as far as population genetics are considered. This study in accordance with (Balakrishna et.al, 2000, Aswathi et. al, 2004; Suryanarayan et.al, 2002; Chikkaswamy et.al, 2007) Fig 3-6.

REFERENCE

1. Akito Kaga,Norihiko Tomooka,Yoshinobu Egawa,Kazuyoshi Hosaka & Osamu Kamijima.(1996)Species relationships in the subgenus Ceratotropis (genus Vigna) as revealed by RAPD analysis. Euphytica 88:17-24.
2. Arvind K Awasthi,GM Nagaraja GV Naik Sriramana Kanginakudru K Thangavelu and Javaregowda Nagaraju **(**2004) Genetic diversity and relationships in mulberry (genus *Morus*) as revealed by RAPD and ISSR marker assays
3. Banerjee, N.S., Manoj, P. and Das, M.R., (1999). Male sex associated RAPD markers in *longum* jCurr. 77 (5): 693-695.
4. Basha, S.D and Sujatha, M. (2007). Inter and intra-population variability of *Jatropa curcas (L.)* characterized by RAPD and ISSR markers. Euphytica. 156:375-386.
5. Chikkaswamy B.K, Rabin Chandra Paramik, Nagaraj Varadaraj H.L.Ramesh, M.Shivashankar and V.Sivaram (2007). Determination of genetic variation in Piper species using 4 C Nuclear DNA and RAPD marker. Int J Cytologia 72 (3): 243-349.
6. Darokar.M.P, Rita Rai, Gupta A.K., Shasany A.K.Rajkumar.S.Sundaresan.V. And Khanuja S.P.S., 2003. Molecular assessment of germplasm diversity in *Aloe* species using RAPD and AFLP analysis.Journal of Medicinal and Aromatic Plant Sciences 25 354-361.
7. Das, A.B., Rai, S and Das, P.1998. Karyotype analysis and 4C DNA content in some species of ginger (Zingiber officinale Rose.). Cytobios 93:175-84.

8. Fabbri .A. Hormaza .J.L and Polito. V.S., (1995). Random amplified Polymorphic DNA analysis of olive (Olea europaea.L.) Cultivars. J. Amer. Soc. Hort. Sci., 120:538-542.
9. Germplasm using RAPD analysis. Indian J. Genet 66: 287 – 292.
10. Girish Naik V., Särkar A., Sathyanarayana N. (2002). DNA Fingerprinting of Mysore Local and V-1 cultivars of mulberry (Morus spp) with RAPD markers.Indian J.Genet, 62(3):193-196.
11. Hormaza, J.L., Dollo, L., and Polito, V.S, (1995). Determination of relatedness and geographical movement of Pistacia vera L. (Pistachio\ Anacondisae) germplasm by RAPD analysis. Econ. Bot, 4: 349 -358.
12. Koller, B., Lehmann, A., Mc Dermott, J.M. and Gessier, C., (1993). Identification of apple cultivars using RAPD markers. Theor. Appl. Genet, 85: 901-904.
13. Krammer, D., Afza, R., Weising, K., Kahl, G., and Novak, F.G. (1992).Oligonucleotide and amplification fingerprinting of wild species and cultivars of banana (Musa spp.). Bio/Technol. 10: 1030- 1035.
14. Naik V.G. Dandin S.B. (2006) Identification of duplicate collections in the mulberry (*Morus* spp.)
15. Nieise, F.P., Hormaza, J.I., and McGranolson, G.H. (1998). Molecular characterization and genetic relatedness among walnut (Jugloss regia L.) genotypes based on RAPD markers. Euphytica. 101:199-206.
16. Plomion, C., Bahrman,N.,Durel,C.E. and Malley,D.M.O.(1995). Genome mapping inpinus pinaster (Maritime pine) using RAPD and protein marker. Heredity 74:661-668.
17. Porebski, S., Bailey, G. and Baum, B.R. (1997). Modification of a C-TAB DNA extra action protocol for plants containing high polysaccharides and polyphenol components. Plant Molecular Biology Reporter, 15, 8-15.
18. Purohit, S.S. and Prajapati, N.D. (2003). Medicinal Plants: Local Heritage with Global Importance, *AGROBIOS News Let*, 1(8) 7-8.
19. Souframanien. J and Gopalakrishna.T (2004). A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theor. Appl. Genet. 109:1687-1693.
20. Suryanarayana, N. (2002) Indian Silk, 41(7):11-13.

21. Vijayan K.Srivastava P.P. and Awasthi A.K., (2004). Analysis of phylogenetic relationship among five mulberry (Monas) species using molecular markers. Genome 47:439-448.

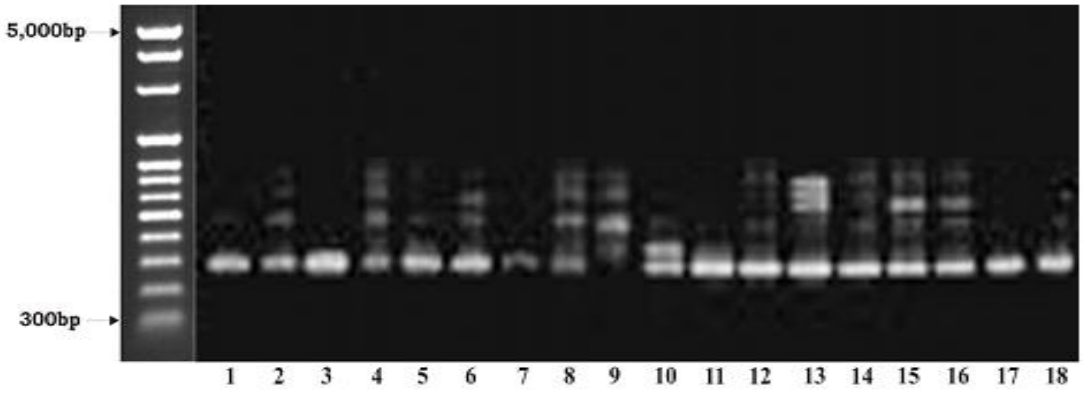

Fig1. Gel profiles of 18 medicinal plants amplified with RAPD Primers –OPAB-05

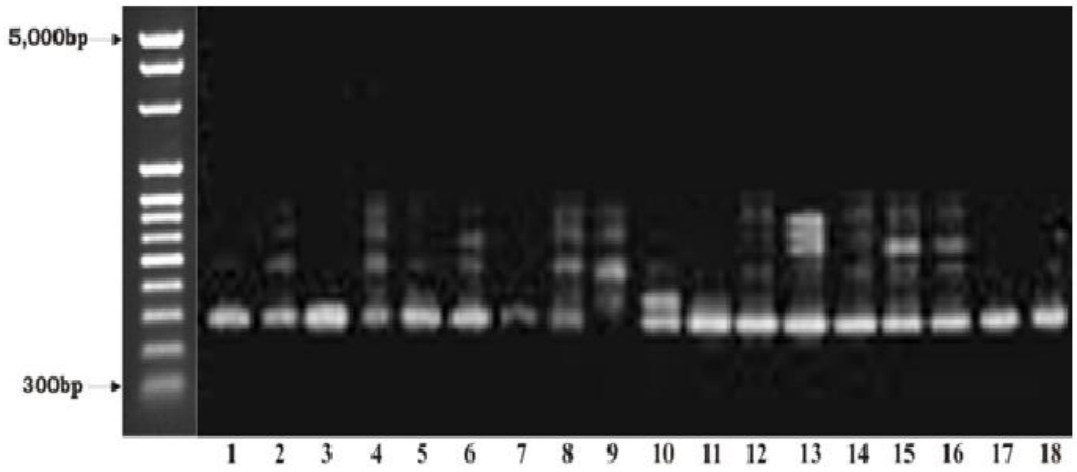

Fig2. Gel profiles of 18 medicinal plants amplified with RAPD Primers –OPAB-014

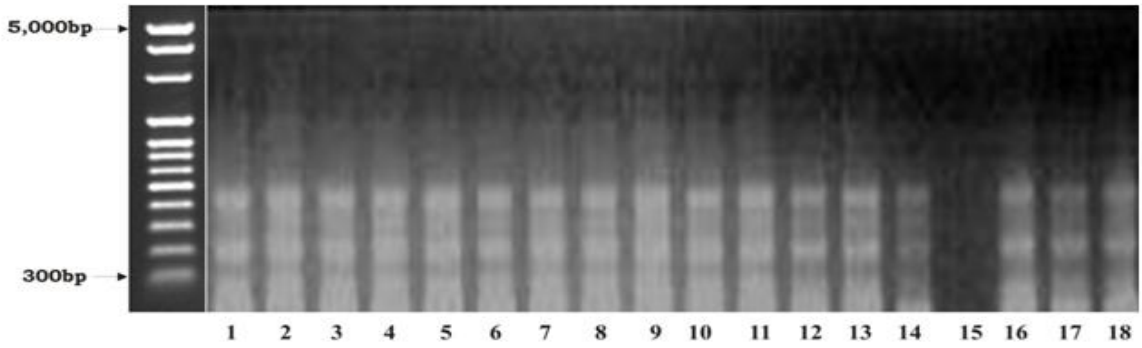

Fig3. Gel profiles of 18 medicinal plants amplified with RAPD Primers –OPAB-05

ISSN: 2278-6244
Impact Factor: 4.054

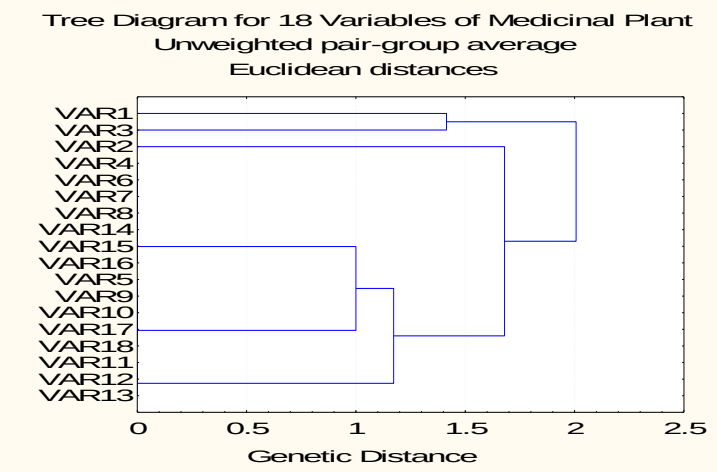

Fig 4. Dendrogram of 18 medicinal plants amplified with RAPD primer- OPAH-13

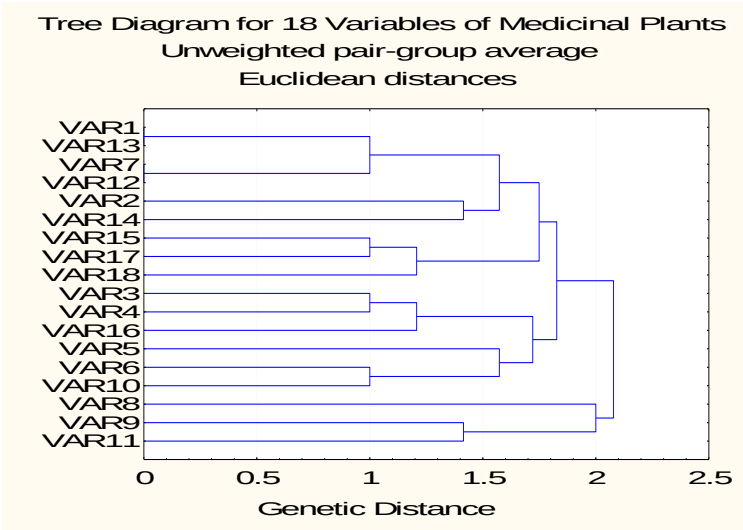

Fig 5. Dendrogram of 18 medicinal plants amplified with RAPD primer- OPAB-14

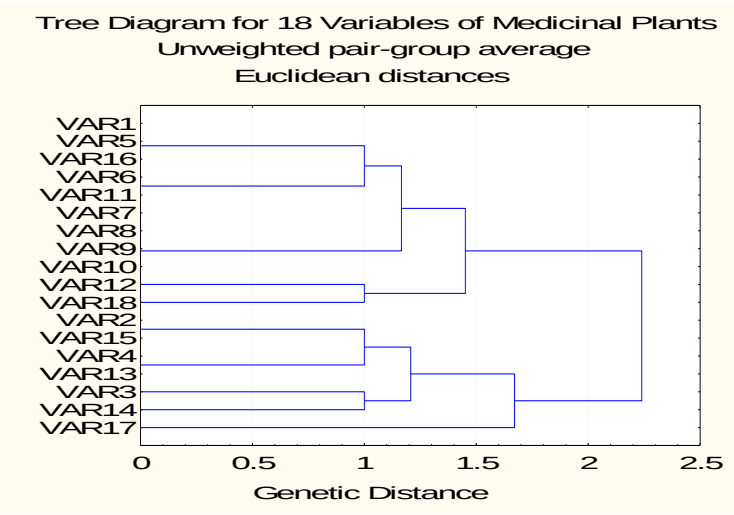

Fig 6. Dendrogram of 18 medicinal plants amplified with RAPD primer- OPAH-13