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***Research Article***

# RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) MARKER ANALYSIS IN *AILANTHUS EXCELSA*

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## ABSTRACT
*Ailanthus excelsa* is a fast growing, indigenous species in India. Understanding the distribution of genetic diversity, among individuals, populations and gene pools is crucial to efficiently manage the variability in germplasm collections for breeding programms. The present paper deals with protocols for isolation of genomic DNA, PCR analysis and its diversity in *Ailanthus excelsa* genotypes from different states of India. This preliminary result indicated total number of amplified products of thirteen primers was 74 and it is the first report of this species. A total of 74 PCR products were obtained and all were polymorphic, Genetic similarity among the species was analyzed by using the software NTSYSpc 2.02 analysis Dendrogram grouped *A.excelsa* which is from different geographical locations together and the Genetic distance values range from 0.27 to 0.65. The primer give the maximum and the minimum level of polymorphism 88% to 40% and it use for the long term breeding programme. This is the first report in Ailanthus excelsa of RAPD marker analysis.

***Key Words:*** *Ailanthus Excelsa, Rapd, Polymorphism and Genetic Distance*

## INTRODUCTION
*Ailanthus excelsa* is a fast growing and an excellent safety matches industry species in India. The genus Ailanthus belongs to the family Simarubaceae consists about 6-10 species. The tree is native to central, western and southern India. It is fairly common in central Sudan and is found planted on reverie and sandy soils. It is indigenous to India, found throughout the drier tropical and sub- tropical parts up to 1000m altitude. It is a large deciduous tree with large pinnate leaves with a rough light grayish bark.
*Ailanthus* is reported to be polygamo-dioecious species with male, female and bisexual flowers (Brandis, 1906; Gamble, 1915 and Nooteboom, 1962). Flowers are small, greenish in colour, arranged in large terminal panicles. The fruit is a flat, membranous samara, dispersed by wind. Chromosome number of the species is (2n=62) (Kumar and Subramaniam, 1986). The tree possesses straight pole and very few branches and attains utilizable girth in 5-6 years. It is very light in weight, soft and not durable and used for packing cases, match box and sticks, fishing floats, catamarans, sword handles and toys (Pearson and Brown, 1932). Apart from being an excellent match wood resource, the species also has several medicinal properties, the bark of the tree is bitter, astringent, that acts as anathematic, febrifuge, antispasmodic, antiseptic and as expectorant. It is also used in treating asthma and bronchitis (Parrotta, 2001).Understanding the distribution of genetic diversity, among individual populations and gene pools is crucial to efficiently manage the diversity in germplasm collections for breeding programms. DNA markers play an important role in understanding the genetic variation in many plant species and they are highly reliable and most preferred choice for germplasm characterization. Currently, several DNA markers are available to assess the genetic diversity of the species. Randomly amplified polymorphic DNA marker (RAPD) is known to be a simple and reliable marker system requiring only small amount of DNA without any knowledge about the genome of the species (Williams *et al.,* 1990 and Waugh and Powell, 1992). At present there are no breeding efforts at the molecular level to improve this species. In this study RAPD markers are used for genetic variation for first time in *Ailanthus excelsa* for its diversity

assessment. In *Ailanthus altissima* genetic relationships was analyzed using other DNA marker system like ISSR and SSR markers. Whereas in *A. excelsa* so far there is no such work was attempted. Here the


**Figure 1: Seed sources from different location of India**

present paper reports RAPD marker analysis of *A.excelsa* in eighteen different seed sources from diverse origin for genetic diversity.

## MATERIALS AND METHODS

### *Plant Material*

Young leaf samples were collected from forty individuals of eighteen different seed sources (Table-1) used as experimental materials and has been established by Institute of Forest Genetics and Tree Breeding at Andhra Pradesh during 2007. All the leaf samples were placed in labeled self sealed poly covers and kept in the ice box until they were transferred in to deep freezer (-20$^0$C).

**Table 1: Details of seed source location in *Ailanthus excelsa***

| S. No | Sample. No | Location |
|---|---|---|
| **1** | 1-5 | Madhya Pradesh |
| **2** | 6-10 | Andhra Pradesh |
| **3** | 11-15 | Rajasthan |
| **4** | 16-20, 40 | Uttrakand |
| **5** | (21,35), (25,26) | Coimbatore, TNPollachi, TN |
| **6** | (22,23), 24, 29 | Salem, TNDharmapuri, TN Kumarapalayam, TN |
| **7** | 27, 28, (30-33) | Palani, TNVirupatchi, TN Theni, TN |
| **8** | 34 | Trichy, TN |

***Isolation of Genomic DNA***

Genomic DNA was isolated from young leaves using the protocol described by Doyle and Doyle (1990) with minor modification. 150 mg leaf tissue was ground to fine powder using liquid nitrogen and suspended in 1 ml of 2 % CTAB extraction buffer (2 % CTAB, Tris-HCl, NaCl, 1% PVPP, 0.1 % B mercaptoethanol). This was incubated at $65^0$C for 1 hr. 1 ml of wet Chloroform and Isoamyl alcohol (24:1) was added and centrifuged at 10,000 rpm for 15 min. RNAse treatment was done in the clear supernatant solution. Then the solution was precipitated with adding of 1 ml of chilled Isopropane-1-al and kept at $-20^0$C for overnight. The precipitated pellet was obtained after centrifugation at 10000 rpm for 10 min. The DNA pellet was washed with 70% ethanol as wash buffer. It was kept at room temperature for 1 hr and centrifuged. The pellet was dried at room temperature and dissolved in 50 µl of sterile water. The suspended DNA was stored at $-20^0$C until use.

***PCR Amplification and Agarose Gel Electrophoresis***

Thirteen Oligonucleotide 10 mer primers from Operon kit B, C, D, E and M (Operon Technologies, USA) were used for the PCR amplification of extracted DNA samples (Table-2). Amplifications were done in

**Table 2: Name of the primers with size, amplified products and polymorphism**

| S.No | Name of the primer | Number of amplified products | Number of Polymorphic bands | % of polymorphism | Size of the fragments |
|---|---|---|---|---|---|
| **1** | OPB-05 | 9 | 8 | 88 | 490bp-920bp |
| **2** | OPB-12 | 2 | 1 | 50 | 850bp-900bp |
| **3** | OPB-18 | 7 | 5 | 71 | 670bp-980bp |
| **4** | OPC-02 | 8 | 5 | 62 | 700bp-970bp |
| **5** | OPD-05 | 5 | 2 | 40 | 650bp-790bp |
| **6** | OPD-10 | 5 | 4 | 80 | 600bp-810bp |
| **7** | OPD-11 | 7 | 6 | 85 | 600bp-940bp |
| **8** | OPE-07 | 4 | 3 | 75 | 700bp-860bp |
| **9** | OPE-18 | 5 | 2 | 40 | 640bp-840bp |
| **10** | OPE-6 | 10 | 7 | 70 | 500bp-920bp |
| **11** | OPE-4 | 2 | 1 | 50 | 850bp-900bp |
| **12** | OPE-13 | 4 | 2 | 50 | 650bp-720bp |
| **13** | OPM-02 | 6 | 3 | 50 | 700bp-970bp |

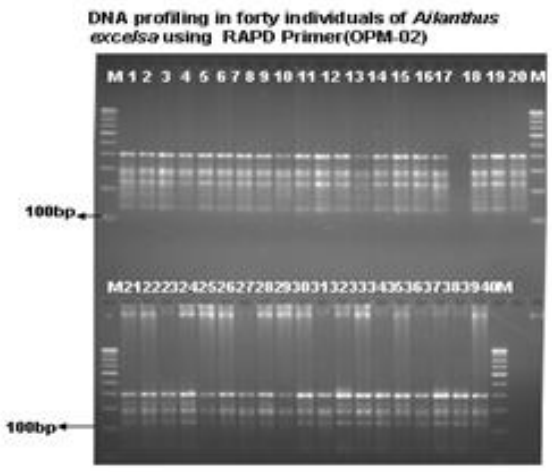


**Figure 2: RAPD primer OPM 02 profiling in *Ailanthus excelsa* for forty different sources**

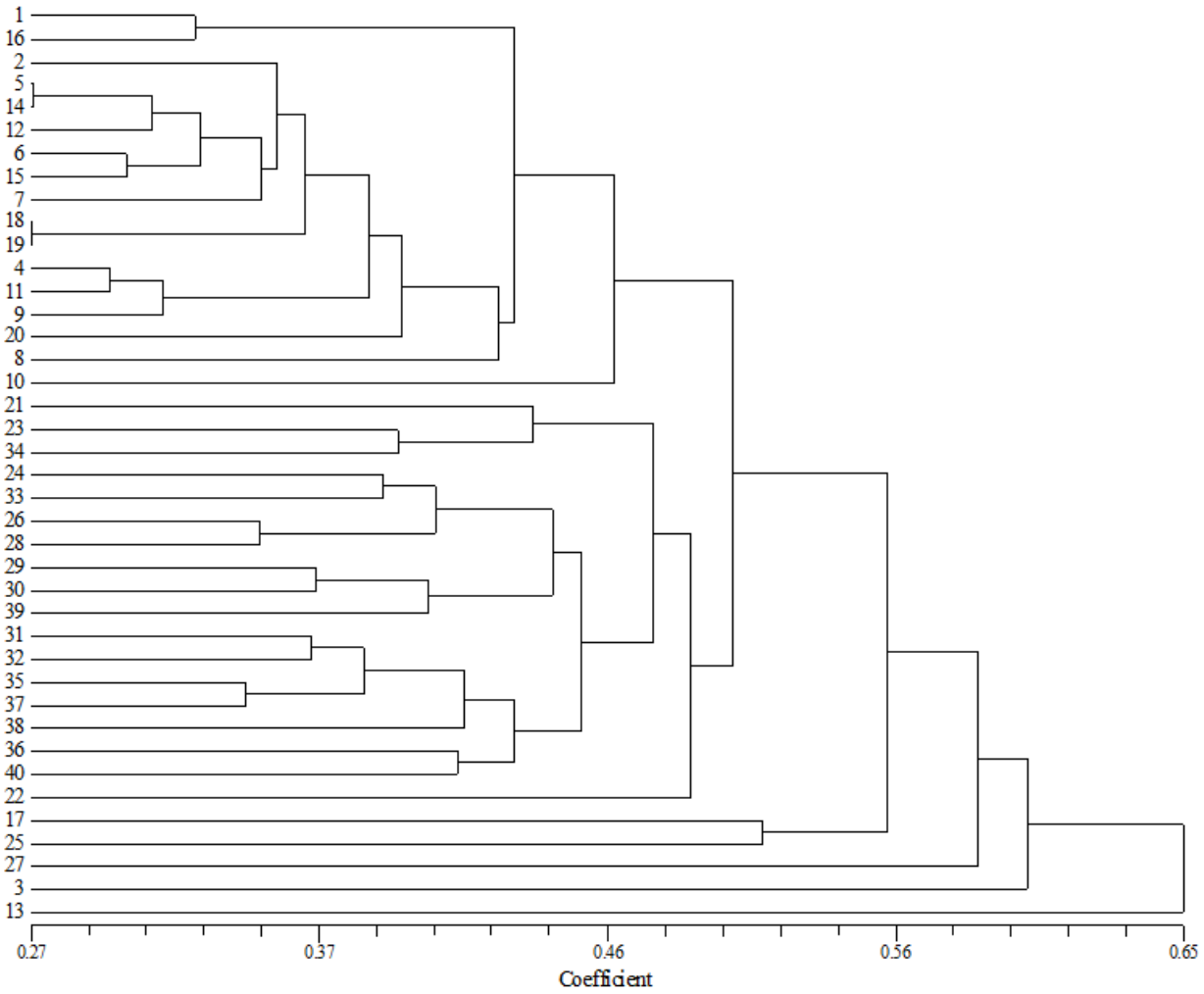


**Figure 3: Genetic distance between the seed sources of *Ailanthus excelsa* based on NTSYSpc 2.02 analysis**

10µl of reaction mixture containing 25ng template DNA, 1 µl of Taw buffer A, 1µl of $MgCl_2$(10mM), 1 µl of dent's (10mM), 0.5 µl of Taw DNA polymerase(3 unit / µl) (Genie, Bangalore). DNA amplification was performed in Eppendorf Thermal Cycler programmed for 36 cycles as follows: 5min at $94^0$C, 1 min at $92^0$C, 1min at $37^0$C and 2 min at $72^0$C followed by one final extension cycle of 5 min at $72^0$C. The amplification products were checked by 1.8% Agarose gel electrophoresis with 1X TBE buffer and stained with ethidium bromide. Consistent and reproducible bands were considered for scoring.

***Data Analysis***

The banding profiles were scored manually for their presence (1) or absence (0) across the genotypes. The binary data matrix table was computed for analysis using NTSYS-pc version 2.1 software**.** The final RAPD data generated with the 13 primers were used to calculate pair wise similarity coefficients (Jaccard 1908) using the Similarity for Qualitative Data format. Cluster analysis was performed on the basis of genetic similarity matrix, and the resulting similarity coefficients were used for constructing dendrogram using the Unweighed Pair Group Method with Arithmetic average.

## RESULTS

Total number of amplified products of thirteen primers was 74. The minimum number of amplified products was 2 in OPB-12 and OPE-4 primers and maximum 10 in OPE-6 primer followed by 9 in OPB-5 primer. Minimum number of polymorphic band was 1 in OPB-12, OPE-4 primers and maximum 8 in OPB-5. The highest percentage of polymorphism in OPB-5 primer was 88% followed by primers OPD-11 (85%) and OPD-10 (80%). All primers were exhibited more than 50% polymorphism except in OPD-5 and OPE-18 have 40% polymorphism. The product sizes of the primers were ranged from 490bp-980bp. The product size of all the primers were indicated more than500bp. Of the 74 products, NTSYSpc 2.02 analysis was carried out and genetic divergence was carried out by using UPGMA cluster analysis. Two major groups were indicated in the dendrogram. Seventeen sources were grouped in one cluster form ( 1, 16, 2, 5, 14, 12, 6, 15, 7, 18, 19, 4, 11, 9, 20, 8, 10). Other groups were formed 21, 23, 34, 24, 33, 26, 28, 29, 30, 39, 31, 32, 35, 37, 38, 36, 40, 22, 17, 25, 1 and 16 indicated. Their genetic distance value was 0.3237 in .5 and14 and also in 18 and 19 are genetically closed. Their genetic distance value was 0.27. The subgroups of 6 and15 (0.3015), 4 and11 (0.2962), 5 and12 (0.2863) were not genetically divergent. The second major cluster has 5 subgroups. The subgroups of 23 and 34 (0.4420), 24 and 33 (0.5156), 26 and 28 (0.3450), 29 and 30 (0.3640), 31 and 32 (0.3624), 35 and 37 (0.3403), 36 and 40 (0.4105), 17 and 25 (0.5118) were formed. Their genetic distance values were indicated within the parenthesis. The genotypes of 13, 3, 27, 22, 38, 39, 21, 2, 7, 9, 20, 8 and 10 did not grouped together and were standing separately. They are highly divergent genotypes. These genotypes can be useful for further improvement programme.

## DISCUSSION

The preservation of genetic diversity within geographical zone having potential economic values is a fundamental goal of conservation strategy. In the present investigation revealed consistent level of genetic variation among the accessions analyzed. The fact that no DNA sequence information is known about this species is one of the main reasons that have motivated the choice of the RAPD technique to develop this study. However, these morphological characters may be unstable and influenced by environmental conditions (Goodrich *et al.,* 1985). Consequently, RAPDs have been intensively used to successfully detect genetic variation between plant populations (Etisham-Ul-Haq *et al.,* 2001), It act as a dominant markers and are inherited in simple Mendelian fashion, therefore, verified as to be taxonomically and evolutionary useful at all taxonomic levels, especially at the intra- and interspecific levels (Demise and Adams, 1994 and Nkongolo *et al.,* 2002). Our present study is used to evaluate the levels of genetic distance within and among populations of *Ailanthus excelsa.* Reports on the analysis of genetic diversity in *A.excelsa*are scanty using molecular markers. Crèche *et al.,* (2000) used RAPD to survey the genetic diversity of *Vaccinium stamineum* within and among patches and found 67 unique RAPD profiles among the 99 sampled individuals from 22 patches. Likewise in A.excelsa 8 different seed sources, 20 randomly selected primers were applied for RAPD fingerprinting analysis and among them 13 resulted in clear and unambiguous consistently reproducible uniform banding patterns. The 13 responding primers resulted in a total of 74 amplification products in the size range of150bp-600bp. Jayram and Prasad (2008) used RAPD markers to assess genetic diversity in *Oroxylum indicum,* a vulnerable medicinal plant collected from 8 locations in Andhra Pradesh, India. High level of genetic similarity was observed in the collected accessions. Overall genetic similarity based on 40 random primers was 87%. The cluster analysis shows two different groups are arranged and it has 62.4% of polymorphic percentage level. Here our present study showed the Tamilnadu seed sources arranged together and other state of Tamilnadu Uttrakhand, Madhya Pradesh, Andhra Pradesh and Rajasthan will arranged in a group. But some of the seed sources which are have the divergence of the genetic distance which could be accompanied as sub groups in the total of the area. Our comparison with other tree species, the gene diversity in *A.excelsa* was comparatively wide range but with relatively higher individual gene diversity values (0.27-0.65) as well

as higher means gene diversity value (0.46). For example Vaishali *et al* also analyzed in *B.monosperma* with higher individual gene diversity values (0.58-0.79) as well as higher mean gene diversity values (0.65). The higher gene mean gene diversity value could be explained since the samples were collected from four agro ecological regions in contrast to the samples of *A.excelsa* collected from different locations. RAPD analysis should prove useful for developing a genetically rich *Ailanthus excelsa* germplasm collection for conservation and for genetic improvement in long-term breeding programs.

***Conclusion***

The preliminary results of this study revealed the genetic diversity in *A.excelsa* genotypes from different regions of India and this was the first report of the DNA marker study of this species

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