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Full Length Research Paper

Molecular analysis of dicot-monocot split and relationship among major angiosperm groups

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The relationship among the major angiosperm groups are modeled based on cladistic analyses primarily using RAPD technique. Phylogenetic trees of relationship derived from molecular data confirm dicots as the ancestral class of monocots, there seems no dicot-monocot split. Dicots form an ancestral class of magnoliids and the monocot lineage was derived from one of the basal magnoliids, since monocots share several synapomorphies dicots do not contain all the descendants of their common ancestor.

Key words: RAPD (Random amplified polymorphic DNA), rRNA (Ribosomal RNA), rbcL (Ribulose 1, 5-bisphosphate carboxylase gene).

INTRODUCTION

Traditionally, the angiosperms were subdivided into two classes, Liliopsida (the monocots) and Magnoliopsida (the dicots) (Cronquist, 1988). However, this subdivision was first refuted by ribulose 1, 5-bisphosphate carboxy-lase gene (rbcL) and 18S rRNA gene phylogenies (Chase et al., 1993; Chaw et al., 1997) and later by ana-lyses of multiple genes from the three plant genomes (Mathews and Donoghue, 1999; Parkinson et al., 1999; Qiu et al., 1999; Soltis et al., 1999; Soltis et al., 2000; Chaw et al., 2000). These phylogenetic analyses have led to the conclusion that the dicots were split into the basal dicots (or the magnoliids) and the eudicots and that the monocot lineage was derived from one of the basal magnoliids. Parallel to the molecular data has been the accumulation of pollen fossils of eudicots, which began in the late Barremian (of Cretaceous, ca. 120 Myr ago) and spread globally in the Albian (ca. 110 Myr ago) (Doyle, 1992; Hughes, 1994). In addition, many new megafossils of basal eudicots have appeared, such as tetracen-traceae from the Barremian (110–118 Myr ago) (Magallo et al., 1999), as well as core eudicots, such as a possible Rhamnaceae / Rosaceae (rosids) from the early Ceno-manian 94 – 97 Myr ago (Basinger and Dilcher, 1984). It has also been suggested that the date of diversification of core eudicots was underestimated. Wikstrom et al. (2001) have examined this issue with nuclear 18S rDNA and two cp (rbcL and atpB) genes.

We now provide additional evidence for dicot-monocot split and origin of core eudicots by analyzing total DNA using RAPD technique. Random Amplified Polymorphic DNA (RAPD) technique (Williams et al., 1990; Welsh and McClelland, 1990) is a frequently used tool in population and evolutionary genetics, since no prior knowledge of the genome structure or sequence data is required. Many studies have successfully applied RAPD analysis in mapping strategies (Martin et al., 1991; Paran et al., 1991; Arnold et al., 1991; Echt et al., 1992; Welsh et al., 1991), strain identification and genomic fingerprinting (Welsh et al., 1991; Koller et al., 1993; Tibayrenc et al., 1993; Halward et al., 1991; Wilde et al., 1992.

MATERIALS AND METHODS

DNA fingerprinting was carried out between angiospermic plants belonging to different genus, plant material was collected from Indian subcontinent, one group of angiosperms comprise of mono-cots as *Curcuma longa* family Zingiberaceae, *Asparagous race-mosus* family Liliaceae, *Commiphora mukul* family Burman-niaceae, *Piper nigrum* family Piperaceae, the other group com-prises of dicotyledonous plants as *Withania sominifera* family.

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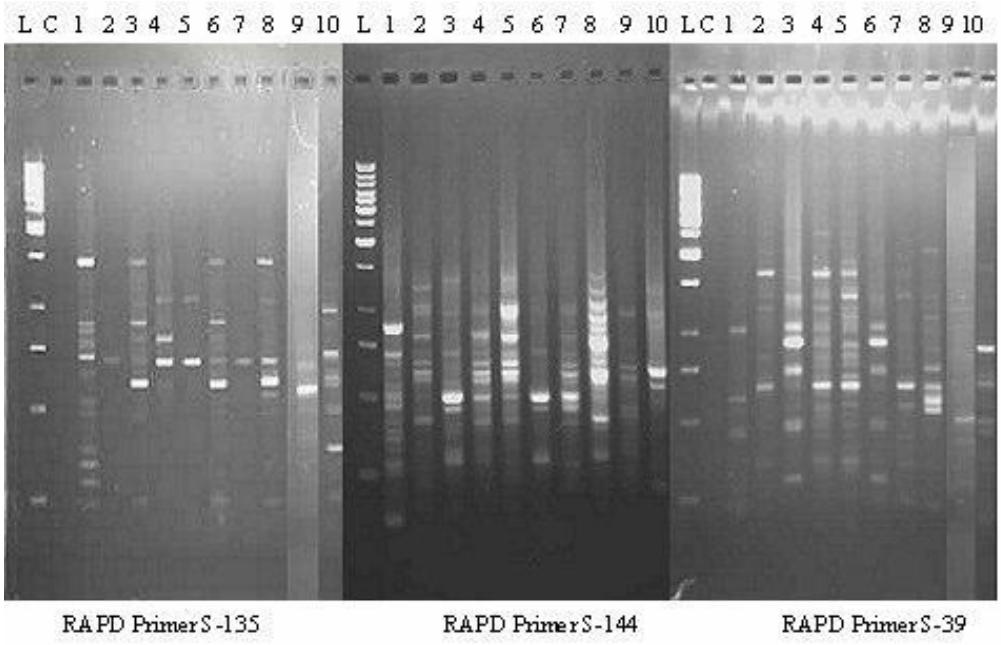

Figure 1. Showing DNA Fingerprint of ten plant genera in PCR-RAPD assay

L = 1kb ladder (Fermentas) C= Negative control Lane 1 = *Cumium cymium* Lane 2 = *Curcuma longa* Lane 3 = *Tribulus terrestris* (VS-1) Lane 4 = *Commiphora mukul* (MH-1) Lane 5 = *Commiphora mukul* (A-1) Lane 6 = *Tribulus terrestris* (C-6) Lane 7 = *Cumium cymium* Lane 8= *Commiphora mukul* Lane 9 *Piper nigrum* Lane 10 = *Withania sominifera*.

Solanaceae, *Cumium cymium* family Cyprinidae, *Tribulus terrestris* family Zygophyllaceae while as *Tinospora cordifolia* family Menispermaceae is placed in the Claude eudicot (APG II system, 2003).

DNA was isolated from 300 mg leaf tissue, in an extraction buffer containing 10% SDS (Sodium dodecyl sulphate) the slurry was incubated at 85^{o}C for 30 min. The lysate was extracted once with equal volume of phenol and Chloroform (1:1 ratio) followed by Chloroform and Isoamyl alcohol (24:1 ratio) at 10,000 rpm for 15 min DNA was precipitated with 2/3 volume of ice cold isopropanol at –20^{o}C overnight, the precipitate was centrifuged at 12000 rpm for 15 min, DNA pallet obtained was washed twice with 70% ethanol dried at 35^{o}C and dissolved in TE buffer pH 8 (Ahmad et al., 2004). PCR reactions were performed in 20 µl reaction volume containing 16ng of template genomic DNA, 10 pM primer, 2 mM of dNTP, 10X PCR buffer with $MgCl_2$, 0.1% BSA and 1U of Taq DNA polymerase. Amplification was performed by first denaturing the template at 94^{o}C for 3 min followed by 34 cycles of amplification with initial denaturation at 94^{o}C for 30 s, 1min annealing at 35^{o}C, 1 min extension at 72^{o}C, with the terminal extention step at 72^{o}C for 7 min, the reaction products were fractionated by 1.5% agarose gel electrophoresis in 1X TAE buffer at 5 V/cm and photographed under UV light with the help of an Image Master VDS (Amersham Biopharmacia, USA). A Gene Ruler^{TM} 1kb DNA Ladder (Fermentas Inc., USA) was used as the molecular standard. Each band was scored for the presence (+) or absence (-) across all the genera, the matrix was subjected to unweighed pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) to generate a dendrogram, using a average linkage procedure all computing was carried out using NTSYS-pc software (Rohlf, 1993). Out of the 180 primers used for the initial screening only three primers (S-135) 5/ CCAGTACTCC 3/ , (S-39) 5/ CCTCTAGACC 3/, (S-114) 5/ GTGACATGCC 3/, revealed amplified reproducible bands among all the genera under study (Figure 1).

RESULT AND DISCUSSION

Phylogenetic studies of angiosperms based on RAPD technique to find dicot-monocot split and genetic divergence rates among different lineages of angiospermic plants indicate clearly that while the monocots form a clade all of the dicots do not form a distinct group separate from the monocots (Figure 2). Instead, the monocots are imbedded in a clade of early branching lineages of flowering plants, usually referred to as magnoliids, all of which have the characteristics of the traditional dicots (Ray, 1703) . These early branches of angiosperms, including the monocots, are characterized by pollen types that are derived from this single-aperture form (Cronquist, A. 1981). Gulnel (*Tinospora cordifolia* V-3) is a eudicot (APG II system, 2003) and forms a distinct clade separate from monocots and dicots (Figure 2). Eudicots are characterized by pollen grains that typically possess three apertures no other morphological or anatomical structures that mark this group have been identified, although the grouping of the eudicots is strongly supported by analyses based on DNA sequence data (Soltis and Soltis. 1999). Our results confirm all previous conclusions proposed by Ray (1703) who first identified the monocots as a group, based largely on their possession of a single cotyledon. These findings indicate that there is no dicot-monocot split instead dicots do not form a monophyletic group (they do not contain all the descendants of

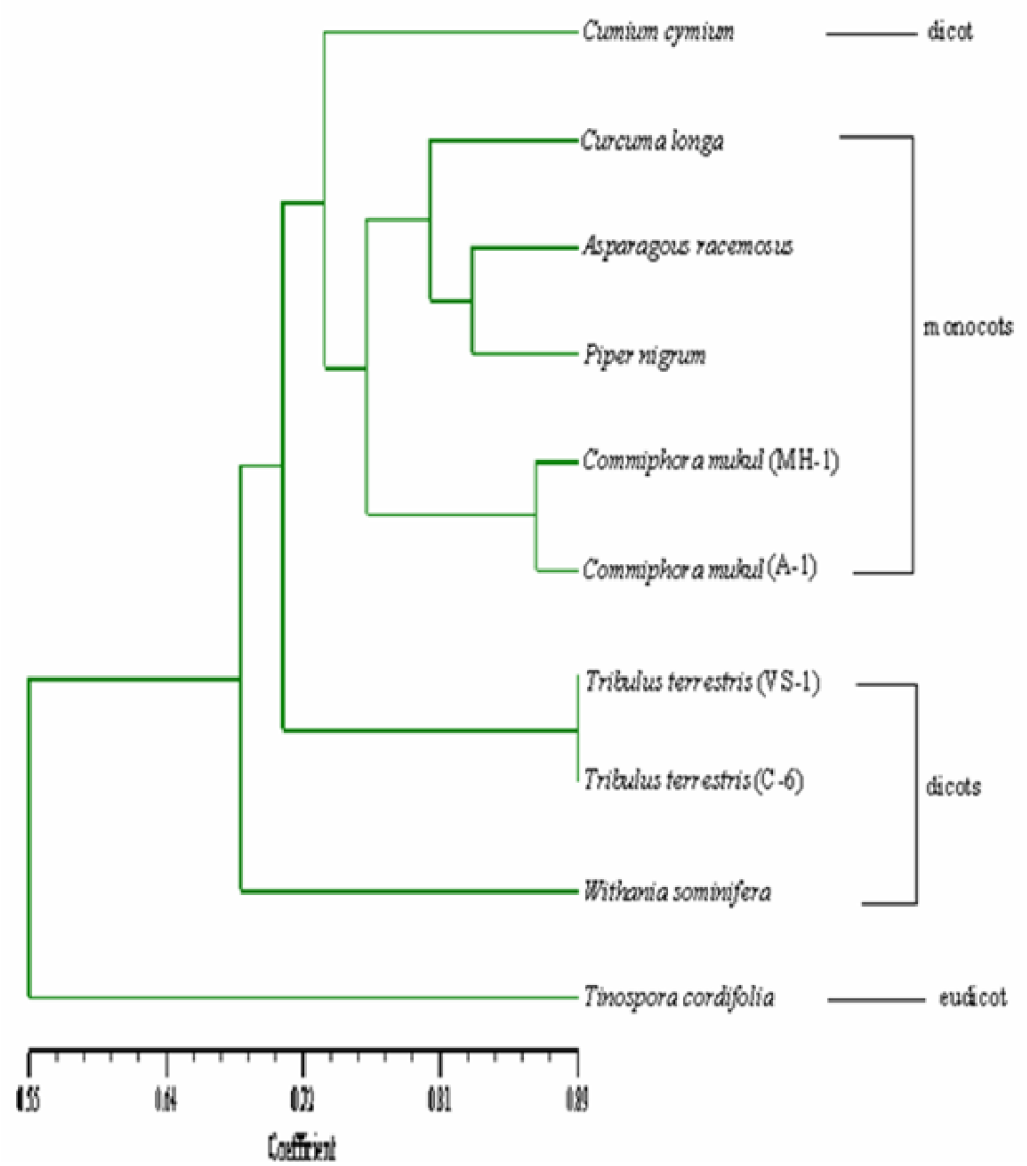

Figure 2. Rooted phylogenetic tree showing genetic relationship among major angiosperm groups belonging to different genera based on UPGMA clustering algorithm.

their common ancestor) and are rejected as a formal group, some dicots are more similar to monocots than they are to other dicots are paraphyletic, monocots can be defined by several synapomorphies whereas the eudicots form a monophyletic group.

Mutations accumulate at constant rate throughout living systems (molecular clock hypothesis), in our study it can be observed that the same is true for rapidly evolving markes (deletions and duplications in the genome) detected by a RAPD technique.

Conclusion

Botanists long theorized that the monocots were derived from an ancient group of dicots during the early diversification of the angiosperms (Donoghue and Doyle, 1989). Phylogenetic trees of relationship derived from molecular data based on RAPD technique confirm this longstanding hypothesis and pinpoint dicots the possible close relatives of the monocots.

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