Summary and Conclusion
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For the biodiversity rich developing countries in the tropics, chemical and genetic prospecting of their plant genetic resources is a priority area not only to fish out genotypes/molecules of potential economic importance but also to add value to them. The present study essentially directed towards this objective, involves analysis of intraspecific variations in *Andrographis paniculata* Nees (Acanthaceae), an important medicinal plant of India, presumably having its centre of origin and diversity in southern India and Sri Lanka. Specifically, the study is directed to: (i) elucidate biology of *A. paniculata* by understanding distribution, habitat, phenology of flowering and fruiting, cytology, breeding system and seed dormancy/germination of the species; (ii) quantify the amount of genetic variability within *A. paniculata* and its patterns of population differentiation; (iii) assess the significance of inbreeding and gene flow to the level and pattern of diversity presently observed in *A. paniculata*; (iv) test the hypothesis that genetic distance is considerably correlated with geographical distance; (v) identify superior genotype(s) with increased biomass and product (andrographolide) synthesis; and (vi) suggest conservation and utilization/improvement strategy for the species based on the data generated.

As part of the study, over 50 populations were sampled from different parts of Asia, of which 17 were short-listed based on phenotypic, genotypic and geographical criteria. Of the selected populations, 15 raised under uniform conditions of growth were analysed at morphological,
cytological, biochemical, phytochemical and DNA levels to reveal extent of intraspecific variability and to possibly select a superior genotype with increased biomass and product (andrographolide) synthesis.

Studies on biology of the species revealed that the plant is naturally self-pollinating, but cross-pollination could not be entirely ruled out. Ten characters were analysed to find out phenotypic variation of the selected populations of *A. paniculata* grown under identical conditions. Considerable diversity in plant height, number of branches, number of leaves, number of flowers, etc. were observed between the populations.

Andrographolide (C_{20}H_{30}O_{5}; MW 350.44), the plant-specific bicyclic diterpenoid lactone is intensively bitter showing a wide spectrum of biological activity. Methanolic extracts of the leaf samples were analysed spectrophotometrically at 223 nm to determine the concentration of the compound following the protocol of Handa and Sharma (1990). Andrographolide content on dry weight basis was highest in AP36 (1.47%) with a mean value of 0.95% for all the populations. Product concentration did not correlate with allelic variations in any of the populations but AP36, with the highest concentration (in the leaves averaged through three generations) may be a prospective cultivar in the present form or after upgradation.

Seed progenies of 15 populations (as germplasm accessions or *ex situ* populations) were analysed at 15 loci representing 8 enzyme systems. On an average 5.4 loci (0.36) were found to be polymorphic (*P*) and mean observed number of alleles per locus (*A*) was 1.41. Average observed heterozygocity (*H_o*) was 0.406 and expected value (*H_e*) was 0.233 showing moderate levels of genetic variation among different populations. The genetic diversity of the 15 populations was also investigated using random amplified polymorphic DNA (RAPD) assays. Overall, 0.82
polymorphism was obtained using 10 primers. The estimated genetic identity measures ranged from 0.343 to 0.943. The UPGMA dendrogram of the 15 populations yielded 4 main groups, which was a reflection of genetic as well as geographical variation.

To study the extent of natural genetic variation, two populations were collected from their habitats and subjected to diversity analysis. Population I from Sirumalai near the eastern spur of the Western Ghats (Sirumalai, Tamil Nadu) consisted of 9 local populations having 24 subpopulations whereas the population II from Eastern Ghats (Nallamalai, Andhra Pradesh) consisted of 2 local populations with 12 subpopulations. The isozyme analysis revealed that the Sirumalai population was genetically more variable (effective number of alleles per locus \( (n_e) \), 1.40; Shannon Information Index \( (I) \), 0.28; Nei’s expected heterozygocity \( (h) \), 0.20; proportion of polymorphic loci \( (P) \), 0.37) than Nallamalai plants \( (n_e, 1.24; I, 0.17; h, 0.13; P, 0.16) \). The RAPD analysis of the two natural populations also showed slightly higher level of genetic diversity estimate for Sirumalai populations (gene diversity \( (h) \)- 0.28; \( I \)- 0.40) compared to Nallamalai population \( (h- 0.25; I- 0.35) \).

RAPD assays with revealed the highest polymorphism among the three kinds of markers (quantitative (morphological/phytochemical), isozyme and RAPD) tested. Overall, twenty-five random primers generated an average of 1.61 bands (alleles) per primer (locus) with a mean polymorphism of 0.71. Genetic distance and diversity estimates derived from isozyme analysis were low in many cases compared to that of RAPD (Sonnante et al., 1997). The present study also pointed to a similar conclusion. With the isozyme analysis, the mean observed number of alleles per locus was 1.37 and the proportion of polymorphic loci was 0.32.
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Genetic differentiation estimated by Nei's distances suggested a common gene pool for the populations collected from Kerala while those from Tamil Nadu and elsewhere from India revealed increased divergence. Exotic populations, more or less exhibited similarity to Tamil Nadu populations which may shed light on their origin. Unlike isozyme analysis, studies based on RAPD will bring even selectively neutral DNA variation into picture and hence an effective correlation between dendrograms obtained from both studies is a difficult task (Sonnante et al., 1997). However, in general, the genetic clustering derived from isozyme and RAPD data was in agreement with the geographical locations/distances.

Further analysis of the two natural populations (Sirumalai and Nallamalai) collected from geographically isolated areas using Nei's distance and fixation indices point towards substantial divergence at the subpopulation level. The results suggest varied drift and bottleneck effects and differential gene flow between them. Local isolation, either natural or artificial, may therefore promote divergence of the populations. Further more, the within-population diversity was significantly different between various populations, indicating that evolutionary potential is not evenly distributed amongst populations.

In conclusion, the present study throws new light on the nature of intraspecific variations in an important medicinal plant (A. paniculata) which have seldom been a subject of investigation. The genetic distances were moderate among different populations, which substantiated previous report on the RAPD analysis of this plant (Padmesh et al, 1998). Unlike a genetically depauperate population facing threat from extinction due to its reduced genetic variability and increased susceptibility to agents of stress (Ledig, 1986), A. paniculata otherwise widely distributed faces threat of genetic depletion from over-exploitation due to its wide popularity as a drug to treat a variety of ailments. It is imperative that measures are initiated for
the conservation of this species with its varied diversity as otherwise potential variants with highest productivity to be developed as cultivars may disappear once for all. However, regarding the conservation of representative populations of *A. paniculata*, caution must be exercised due to sizeable differentiation at the local and within-population levels. Sirumalai being a rich source of *A. paniculata* but showing populations with only moderate levels of variation presumably due to over collection by poor people of nearby 35 villages needs to be accorded priority in the conservation of the species without further loss of genetic diversity. For practical conservation purpose, maintenance of maximum genetic variation within existing populations and sustainable use by the local communities, outright collection of plants before seed set in a given locality in the Western Ghats region in Kerala and Tamil Nadu or parts of Eastern Ghats in Andhra Pradesh, where plants were sampled, should be discouraged. Although AP36 may prove to be a prospective cultivar, preliminary trials were conducted to upgrade this selection through chemical (EMS) mutagenesis. A presumptive mutant (CM1) so isolated showed somewhat enhanced phytochemical and biomass production qualities which were stable through 3 generations. In the light of the results presented and discussed, it is the well considered opinion of the author that a two way approach viz. selection of an improved natural variant for development as cultivar and isolation of a high yielding cultivar from the selected genotype though mutagenesis would be most fruitful for achieving the twin objectives of conservation of existing genetic diversity and sustainable utilisation of this important medicinal resource.