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Isozyme diversity of *Garcinia gummigutta* (L.) N. Robson in Western Ghat region, South India

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Abstract

Isozyme genetic markers are efficient tools to study genetic variations within and between populations of less known wild species as well as for studies on spatial distribution of genetic variation. A study was conducted with four important isozyme markers namely, peroxidase, polyphenol oxidase, esterase and superoxide dismutase in *Garcinia gummigutta* population collected from Western Ghats in South India. The cluster analysis of the marker bands showed that most of the population from similar geographic locations was the first one to group themselves, though a significant pattern was not noticed. The mean percentage of polymorphic loci was 52.5%. Total heterozygocity was 0.97 which is consistent with the average of tropical tree species.

Keywords: *Garcinia gummigutta*, geographic distance, isozyme markers.

Abbreviations: Em value (Electrophoretic mobility value), PRX (Peroxidase), PPO (Polyphenol oxidase), SOD (Superoxide dismutase), NTSYS (Numerical taxonomy system).

Introduction

About 30 species of the genus *Garcinia*, including *G. gummigutta* (L.) N. Robson are indigenous to Western Ghats and are extensively distributed in the Western Ghat range of Kerala and Karnataka. Western Ghats with its varied environmental conditions is one of the important hotspots of biological diversity including plant species.

Isozyme genetic markers have been used for many decades and are still one of the most reliable and efficient tools in estimation of heterozygosity and prediction of diversity in plant species (Lewontin & Hubby 1966). In forestry, isozymes have been used to study genetic variation within and between populations. Isozymes are of great use in the elucidation of genetic information for relatively unknown species and it has been used to determine species delimitation (Rajora 1989). Li (1999) found that isozyme variation in *Eucalyptus microtheca* F. Muell has significant pattern related to geographic distance. This environmental variation might also be expected to favour genetic heterogeneity. The aim of the present study was to detect isozyme polymorphism in *G. gummigutta* collected from

different geographical locations in Kerala and Karnataka and provide information on geographical variation that may be responsible for intra-species genetic variation. This information would help in the development of conservation and utilization strategies of genetic resources of *G. gummigutta*.

Materials and methods

Twenty four natural populations of *G. gummigutta* were studied. The plants (about 8 years old) were collected from various districts of Kerala and Karnataka located in the Western Ghats range. The youngest matured leaf from the plants were used for the study. The leaves were macerated in 1000 µl of sodium phosphate extraction buffer of pH 7.2 in porcelain mortar over ice (Sadasivam & Manikam 1992). The vertical gel buffer electrophoresis (ATTO) system was used for the separation of four isozymes namely, PRX, PPO, esterase and SOD. The running gel (10%) and stacking gel (5%) were of poly acrylamide gel. The loci were numbered from anode to cathode. The cluster analysis was done to construct the polygenetic tree using NTSYS PC2 (Rohlf 1993) software.

Collection sites were plotted on the map with the help of ArcGIS software to verify the similarity among clusters with distance (Fig. 1). Using DIVA GIS bioclim model, the altitude and rainfall grids of the collection sites were prepared to find out their effect on the enzymes if any.

Genetic parameters such as mean number of alleles per locus, percentage of polymorphic loci, observed heterozygocities, genetic distance and similarities were calculated for the studied natural populations. Among the population, heterogeneity tests were done based on allele frequencies and polymorphic index obtained from isozyme analysis (Ashburner *et al.* 1997).

Results and discussion

The number of bands in PPO and PRX varied from 2 to 4. The Em values were almost same

Fig. 1. Map of Kerala and part of Karnataka showing the collection sites

for both the enzymes (Table 1). For esterase and SOD, uniform 2 and 3 bands were noticed in all the samples under study. For esterase, the Em values were 0.717 and 0.871 and for SOD, the values were 0.167, 0.667 and 0.807. The polymorphic index calculated for PRX was 0.0053 and for PPO it was 0.0037. This indicates that the intra-population variation of both the enzymes was not much and the mean percentage of polymorphic loci was 52.5%. The heterogeneity was worked out based on allelic frequency and polymorphic index and was 0.97. Cluster analysis based on NTSYS pc2 revealed low level of genetic separation within the population (Fig. 2). The coefficient values were within 0.85 to 1.00. The resulting dendrogram showed differentiation into three main clusters and

Table 1. Isozyme band numbers and Em values for polyphenol oxidase and peroxidase

Sample	Longitude	Latitude	No. of bands (polyphenol oxidase)/ (peroxidase)	Em value (polyphenol oxidase)	Em value (peroxidase)
Thrissur 1	76⁰30′82″	10⁰53′56″	2	0.5, 0.571	0.421, 0.521
Thrissur 2	76⁰28′82″	10⁰43′56″	2	0.5, 0.571	0.521, 0.62
Thrissur 3	76⁰29′82″	10⁰43′56″	2	0.5, 0.571	0.521, 0.62
Thrissur 4	76⁰27′93″	10⁰53′56″	2	0.5, 0.571	0.421, 0.521
Thrissur 5	76⁰30′82″	10⁰53′56″	2/1	0.5, 0.571	0.521
Thrissur 6	76⁰29′23″	10⁰56′56″	3	0.5, 0.571, 0.642	0.421, 0.521, 0.62
Calicut 1	75⁰78′39″	11⁰69′22″	2	0.5, 0.571	0.421, 0.521
Calicut 2	75⁰80′29″	11⁰64′56″	2	0.5, 0.571	0.421, 0.521
Calicut 3	75⁰77′29″	11⁰65′49″	3	0.5, 0.571, 0.642	0.421, 0.521, 0.62
Calicut 4	75⁰78′29″	11⁰63′49″	3	0.5, 0.571, 0.642	0.421, 0.521, 0.62
Calicut 5	75⁰77′29″	11⁰65′49″	3	0.5, 0.571, 0.642	0.421, 0.521, 0.62
Appangala 1	75⁰77′21″	12⁰26′21″	2	0.5, 0.571	0.421, 0.521
Appangala 2	76⁰00′71″	12⁰06¹21″	3	0.321, 0.5, 0.571	0.295, 0.421, 0.52
Appangala 3	75⁰81′45″	12⁰13′68″	3	0.321, 0.5, 0.571	0.295, 0.521, 0.62
Kannur 1	75⁰44′36″	12⁰07′53″	2	0.5, 0.571	0.521, 0.62
Kannur 2	75⁰49′36″	12⁰04′53″	3	0.5, 0.571, 0.642	0.421, 0.521, 0.62
Kannur 3	75⁰42′36″	12⁰08′53″	3	0.5, 0.571, 0.642	0.421, 0.521, 0.62
Kannur 4	75⁰59′36″	12⁰02′53″	3	0.5, 0.571, 0.642	0.421, 0.521, 0.62
Kannur 5	75⁰49′36″	12⁰04′53″	3	0.5, 0.571, 0.642	0.421, 0.521, 0.62
Wayanad 1	76⁰12′04″	11⁰57′79″	2	0.5, 0.571	0.421, 0.521
Wayanad 2	76⁰08′86″	11⁰76′22″	3	0.5, 0.571, 0.642	0.421, 0.521, 0.62
Wayanad 3	76⁰18′39″	11⁰57′79″	3	0.5, 0.571, 0.642	0.421, 0.521, 0.62
Kottayam 1	76⁰63′12″	9⁰64′25″	4	0.321, 0.5,0.571, 0.64	0.295, 0.421, 0.521, 0.62
Kottayam 2	76⁰76′14″	9⁰64′25″	4	0.321, 0.5, 0.571, 0.64	0.295, 0.421, 0.521, 0.62

seven sub-clusters. The altitudinal and precipitation study of the collection sites threw some light on the differences in the environment and segregation but further study is required for better understanding.

The mean percentage of polymorphic loci which was 52.5%, indicated that genetic variability within the population of *G. gummigutta* was not much higher than out crossing in wind pollinated woody plants which is 53%, and the result is consistent with the average of tropical tree species (60.9%) (Li 1999).

Cluster analysis revealed low level of genetic separation between the studied populations, the coefficient of cluster varied between 0.85 to 1.00. The resulting dendrogram showed differentiation into three main clusters. No significant pattern related to geographic distance could be found. The dendrogram showed that most of populations from similar geographic locations were the first ones which group themselves, but the clustering of Calicut population did not follow any criterion. The Wayanad collections were in separate groups (the distance between

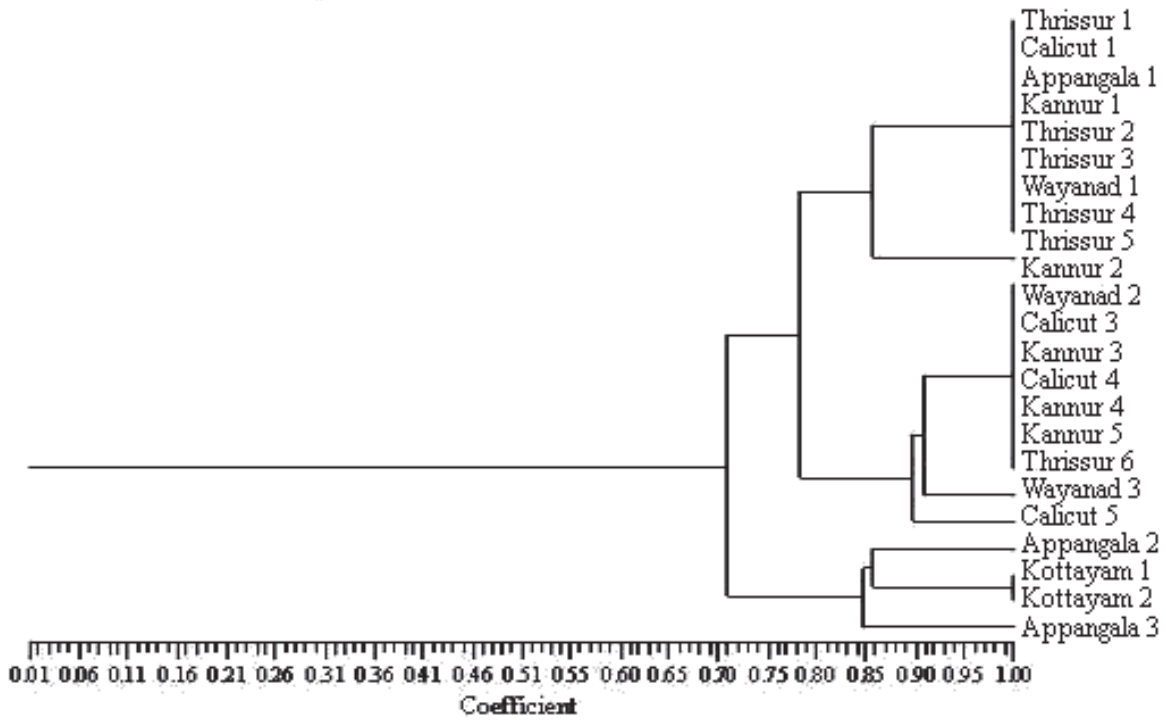

Fig. 2. Similarity clusters based on NTSYS pc2 software (the accessions are named based on their collection sites).

Sugandhagiri and Pulppally is 55 km), while Thrissur collections are in same group except one (separated by10 km). Kottayam (77°6312/ 9°6425 & 77°7614/9°6425) and Appangala (Kodagu) (75°7721/12°2621, 76°0071/12°0621 & 75°8145/12°1368) collections clustered together, but in different sub-groups, though they were collected from nearby geographic locations.

This pattern has also been observed in most of the studied populations of forest trees (Elenarossello & Cabrera 1996). It is important to mention that the cluster analysis and the resulted dendrogram were based only on the information of the allelic frequencies (of four isozymes) of each population but not on any special morphological character in the population. Two sites of Appangala (Kodagu) have the same altitude and rainfall (800-1000 m and above 3000 mm) respectively, and in the cluster they showed segregation.

In forestry, isozymes have been used to study genetic variation within and between populations, population structure, phylogeny, and to elucidate mating patterns among natural populations as well as experimental populations (Mitton 1983; Hamrick & Godt 1989). When a little information about genetic diversity within or between populations is known, isozymes can be an effective genetic marker. In "wild" species like G. *gummigutta* with scanty information on genetic diversity, the need for basic data such as the levels of heterozygosity and per cent of polymorphic markers within population are important. In this study, isozyme data was generated for the comparison of genetic variation within populations three and seven sub-clusters were found within 1000 km distance of Western Ghats. Total polymorphic percentage was 52.5%, which indicated low heterogenicity. Garcinia being a cross pollinated species, it is expected to observe much higher diversity but the result is consistent with the average reported for tropical tree species.

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