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Communication

Variation in physical characters and evaluation of genetic diversity in *Acacia caesia* var. *caesia* (L.) Willd. in an environmental gradient in the Western Ghats of Tamil Nadu, India

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Abstract: A random amplified polymorphic DNA (RAPD) analysis was made to determine the genetic variation between the five populations of the medicinal shrub, *Acacia caesia* var. *caesia* in an environmental gradient. Of the nine random primers tested, one primer, UBC-807 showing polymorphism was used in RAPD analysis. The primer produced 12 scorable DNA fragments in the populations studied, which showed the existence of genetic variation between populations. A dendrogram was constructed based on Jaccard's coefficient to determine the degree of genetic relationship among the five populations and analysed. It showed that the genetic similarity among the populations varied from 55 to 89 %. Further, the genetic polymorphism in the population in a very arid habitat at Chennimalai was higher (70%) than that of other populations, which indicates the plasticity of the genome in a water-stressed environment. Observation of morphological features shows that the population in a very arid habitat had decreased vegetative growth with increased reproductive attributes.

Keywords: *Acacia caesia* var. *caesia*, RAPD analysis, polymorphism, Jaccard's coefficient, morphological variation

INTRODUCTION

Acacia caesia var. *caesia* (L.) Willd. (Leguminosae, Mimosoideae) is a medicinal plant locally used in Coimbatore district of Tamil Nadu, India. The leaves are used for the treatment of bronchitis (asthma), colds and skin problems. It is an armed woody straggling shrub, mainly distributed in tropical regions of India, Sri Lanka and Thailand.

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In India it is abundant in the foothills of the Western Ghats [1] in different environmental conditions with distinct morphological variation. The effectiveness of medicinal properties of certain populations of this species inhabiting dry environments is better than that from other habitats [2-3]. In addition to environmental factor, genetic constitution is also known to contribute to this difference in properties in many species [4-6]. To determine the best population for medicinal properties as well as the level of genetic polymorphism in this plant, random amplified polymorphic DNA (RAPD) analysis was undertaken by using UBC-807 RAPD markers.

MATERIALS AND METHODS

Materials

Five different populations of *A. caesia* var. *caesia* were selected in an environmental gradient situated in the Western Ghats of Western Tamil Nadu, viz. at Chennimalai (very arid; 450 m above msl; thorny-scrub jungle), Maruthamalai (arid; 540 m above msl; dry deciduous forest), Palamalai (semi-arid; 620 m above msl; moist deciduous forest), Thadagai Hills (humid; 680 m above msl; moist semi-evergreen forest) and Burliar (very humid; 760 m above msl; moist evergreen forest).

DNA Isolation and Primer Screening

Young tender leaves were collected from 50 individuals at each population and were washed with double distilled water and stored in sealed polythene bags at -70^{o}C in a deep freezer until use. Genomic DNA was isolated from 100 mg of the leaf tissue by using GenElute plant genomic DNA purification kit (Sigma-Aldrich) and following the manufacturer's instructions.

Quality Check and Quantification of Genomic DNA

About 2 µl of the genomic DNA isolated from 100 mg of leaf tissue was subjected to electrophoresis on a 0.8% agarose gel containing 0.5 µg/ml of ethidium bromide. After electrophoresis, the gel was viewed over a UV transilluminator (UVT-40M, Herolab) and the quality and quantity of the DNA were assessed by using undigested λ DNA as a control. The genomic DNA was diluted to 4ng/µl and stored at 4^{o}C as a working solution while the stock DNA (undiluted) was stored at -20^{o}C in aliquots.

RAPD Polymerase Chain Reaction (PCR)

The RAPD polymerase chain reaction (PCR) was carried out in 20-µl reaction volume containing genomic DNA (28 ng), *Taq* DNA polymerase (1 unit), 0.5 µM primer 0.2 mM dNTPs, 10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl2, 50 mM KCl and 0.01% gelatin. Amplification was carried out in a thermocycler (Eppendorf) with an initial strand separation at 94^{o}C for 4 minutes, followed by 40 cycles of amplification and final extension for 1 minute at 37^{o}C and 1.5 minutes at 72^{o}C. After 40 cycles, there was a final extension step of 5 minutes at 72^{o}C. A negative control without genomic DNA template was used for amplification along with genomic DNA from 5 habitats with primers. This was done to confirm the quality of primer dimers or possible contaminants. Amplification products were

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resolved on 1.2% agarose gel in 1 X TBE buffer stained with ethidium bromide (0.5 µg/ml). The gels were photographed using a gel documentation system (Syngene).

Observation on Morphological Characters

To observe morphological variation for plant shoot length, root length, number of lateral roots, stem girth, number of branches, number of leaves, leaf thickness, leaf surface area, number of inflorescences and number of fruits, a hundred mature individuals in each population were randomly selected with three replications.

Molecular Characterisation

Each RAPD band was treated as a unit character and was scored manually as independent binary codes ('1' for presence and '0' for absence). Only distances and well-resolved bands were scored. The bands that had conflicting data between the two readings were eliminated from further analysis. The percentage of polymorphism was calculated as the proportion of polymorphic bands to the total number of bands. The 1/0 matrix was prepared and the data were used to generate genetic similarity (GS) based on Jaccard's coeffecient of similarity: GS (ij) = a/a+b+c, where GS (ij) is the measure of genetic similarity between individuals i and j, a is the number of polymorphic bands that are shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. To examine the genetic relationship among populations, a dendrogram was generated from distance values using the unweighted pair-group method of arithmetic averages (UPGMA), using the multivariate statistic package (MVSP) software version 3.13n [7].

RESULTS AND DISCUSSION

DNA extracted from the five populations was examined for their PCR-RAPD patterns. Out of 9 primers screened, one was selected based on robustness of amplification, reproducibility and scorability of banding pattern and used for diversity analysis in all five populations. The UBC-807 AGAGAGAGAGAGAGAGT primer generated 12 amplification products and out of them 7 bands had 58.3% polymorphism (Figure 1). The number of polymorphic bands ranged from 4 to 7 across the populations analysed and a total number of 12 polymorphic bands were formed in the five populations. The percentage of polymorphism obtained in the populations varied. In Chennimalai there was 70% polymorphism followed by 62% in Maruthamalai, 60% in Burliar, 55% in Palamalai and 50% in Thadagai Hills (Tables 1-2).

The Jaccard's coefficient of genetic similarity matrix [8] prepared on the basis of RAPD data showed that it varied from 0.55 to 0.89 among the clusters–Maruthamalai to Burliar through Thadagai Hills, and Palamalai and Chennimalai (Figure 2). It showed that abiotic environmental factors influenced genetic differentiation within and between populations [4]. Also important to note is the chance of sharing a common gene pool between all populations studied except Chennimalai population which has a higher percentage of polymorphism. Populations at Chennimalai and Burliar, which are at two extremes in the environmental gradient, generally showed high degrees of polymorphism, which may be explained due to the different environments. The lower levels of polymorphism in the other populations

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is due to the expansion within the range of variation. The genetic diversity evidence from the RAPD analysis clearly indicates the interaction between a major abiotic factor, i.e. climate, and other minor abiotic factors in shaping the genome to survive in other environments.

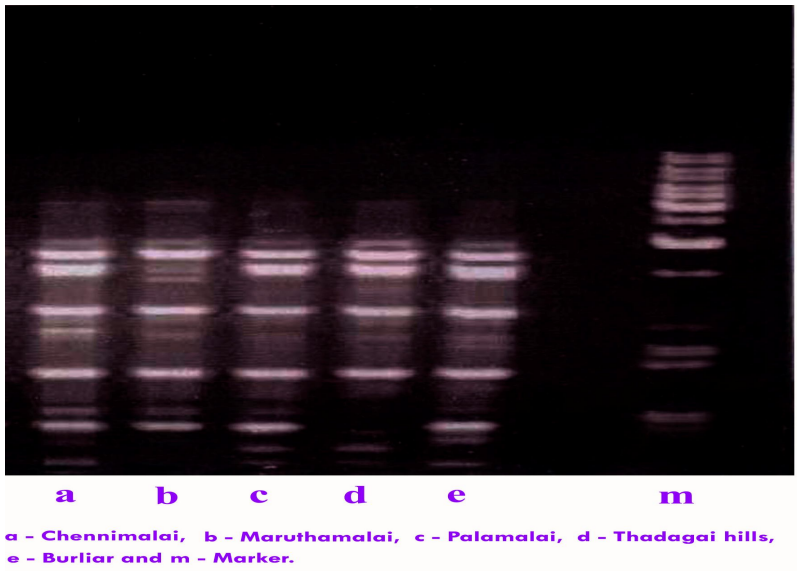

Figure 1. Amplification products obtained from DNA of the five different populations of *A. caesia* with UBC-807 AGAGAGAGAGAGAGAGT primer

Table 1. Matrix coded for bands obtained in RAPD analysis in *Acacia caesia*

Population	Band											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Chennimalai	1	1	1	0	1	1	1	1	1	1	0	1
Maruthamalai	1	1	1	0	1	1	0	1	0	0	1	1
Palamalai	1	1	1	0	1	0	0	1	1	1	1	1
Thadagai Hills	1	1	0	1	1	1	0	1	1	1	0	0
Burliar	1	1	1	0	1	1	0	1	1	1	1	1

Note: 1 - Presence of band; 0 - Absence of band

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Table 2. Polymorphic and non-polymorphic bands for UBC-807 AGAGAGAGAGA GAGAGT primer in *A. caesia* populations

Population	Total no. of bands	Polymorphic bands	Percentage of polymorphism
Chennimalai	10	7	70
Maruthamalai	8	5	62
Palamalai	9	5	55
Thadagai hills	8	4	50
Burliar	10	6	60

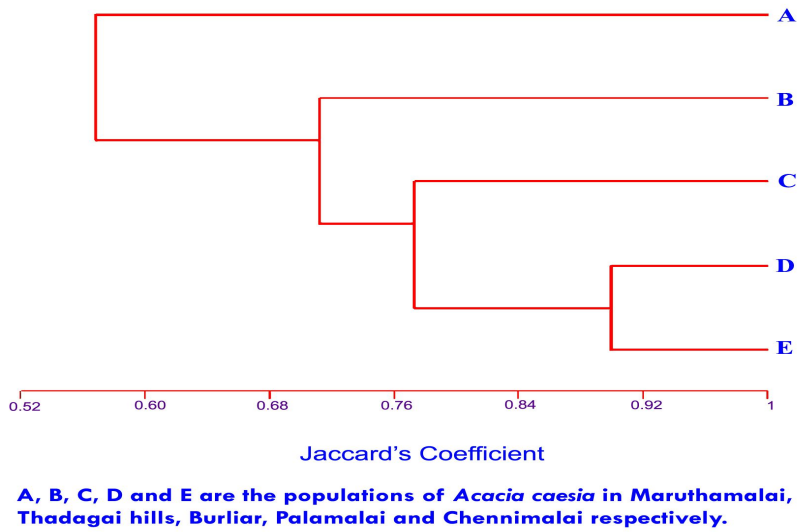

Figure 2. Dendrogram for the five populations of *A. caesia* developed from RAPD data using unweighted pair-group method of arithmetic averages (UPGMA)

It is known that vegetative traits of this plant, e.g. shoot length, root length, number of lateral roots, stem girth, number of branches, number of leaves, leaf thickness and leaf surface area, generally increase with more humid conditions (Table 3). Reproductive attributes such as the number of inflorescences and number of fruits per plant are more in arid condition at Chennimalai and Maruthamalai. It indicates that the suppression of vegetative growth in arid places concomitantly enhanced the reproductive ability of the species. This feature was observed by Patricia et al. [9] for *Grevillea barklyana* and by Chandler and Bartels [10] for many wild plants.

Sathishkumar et al. [2] reported that the medicinal value was significantly higher in the population of *A. caesia* in the very arid habitat at Chennimalai. The high degree of polymorphism (70%), higher expression of reproductive characters and greater medicinal value at Chennimalai indicates the best area for cultivation of this species. Pharmacognostical studies are needed to confirm this observation.

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Table 3. Morphological and reproductive features of *A. caesia* studied at the five sites

Character	Study area*				
	Chennimalai (very arid)	Maruthamalai (arid)	Palamalai (semi-arid)	Thadagai Hills (humid)	Burliar (very humid)
Shoot length (cm)	$228.84^{a} \pm 5.38$	$244.16^{b} \pm 4.02$	$243.02^{b} \pm 8.49$	$249.70^{c} \pm 6.36$	$250.36^{c} \pm 4.02$
Root length (cm)	$149.38^{a} \pm 4.57$	$151.70^{a} \pm 2.46$	$150.74^{a} \pm 5.27$	$159.08^{b} \pm 2.36$	$159.60^{b} \pm 2.46$
No. of lateral roots/ plant	$78.90^{a} \pm 1.83$	$80.70^{a} \pm 2.94$	$82.18^{b} \pm 2.85$	$85.78^{c} \pm 2.94$	$86.50^{c} \pm 2.94$
Stem girth (cm) / plant	$3.90^{a} \pm 0.57$	$4.17^{b} \pm 0.65$	$4.14^{b} \pm 0.29$	$4.86^{b} \pm 0.68$	$4.79^{b} \pm 0.65$
No. of branches /plant	$14.52^{a} \pm 1.88$	$18.08^{b} \pm 3.16$	$17.78^{b} \pm 4.08$	$23.14^{c} \pm 3.16$	$23.80^{c} \pm 3.11$
No. of leaves / plant	$2594.94^{a} \pm 0.20$	$3175.06^{b} \pm 10.83$	$3198.86^{b} \pm 9.60$	$3302.24^{b} \pm 10.83$	$3314.24^{b} \pm 12.42$
Leaf thickness (mm)	$0.33^{a} \pm 0.02$	$0.35^{a} \pm 0.03$	$0.34^{a} \pm 0.02$	$0.38^{a} \pm 0.01$	$0.38^{a} \pm 0.01$
Leaf surface area (cm^2)	$18.15^{a} \pm 0.64$	$17.05^{a} \pm 0.33$	$17.03^{a} \pm 1.71$	$20.17^{b} \pm 0.33$	$20.19^{b} \pm 0.33$
No. of inflorescences/plant	$135.10^{a} \pm 1.67$	$133.58^{a} \pm 3.46$	$133.28^{a} \pm 2.33$	$132.20^{a} \pm 3.46$	$131.70^{a} \pm 4.34$
No. of fruits /plant	$92.04^{a} \pm 2.50$	$89.88^{b} \pm 4.36$	$89.42^{b} \pm 3.07$	$86.12^{c} \pm 4.36$	$85.02^{c} \pm 4.41$

Note: In horizontal rows, means followed by different letters are significant to each other at 5% level according to DMRT.

* Chennimalai – 450m above msl, thorny scrub jungle, hard stony soil; Maruthamalai – 540m above msl, dry deciduous forest, graval with shallow soil; Palamalai – 620 m above msl, moist deciduous forest, coarse sand with gravels; Thadagai Hills – 680 m above msl, moist semi-evergreen forest, sandy loam and shallow soil; Burliar – 760 m above msl, moist evergreen forest, humus soil with shallow nature

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