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Research Article

ASSESSMENT OF GENETIC DIVERSITY OF ANTI-CANCER DRUG YIELDING PLANT *ANDROGRAPHIS PANICULATA* NEES FROM THE WESTERN GHATS OF TAMIL NADU USING ISSR MARKER

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ABSTRACT

In the present investigation the genetic diversity of anti-cancer drug yielding plant *Andrographis paniculata* Nees collected from the Western Ghats of Tamil Nadu was carried out using ISSR- PCR makers. After screening twenty ISSR primers, 10 intense and reproducible bands produced primers were selected for the study. ISSR markers amplified products produced a total of 50 loci in ten primers at ten different populations of *Andrographis paniculata*. High genetic variation at species level was observed in the present investigation with the recording of 96 percentages of polymorphic bands (PPB) and with the 48 number of polymorphic loci. The mean value of overall observed and effective number of alleles were 1.96 and 1.6435. Nei's gene diversity and Shannon information index were 0.3697 and 0.5446. The mean value of genetic diversity within population (H_s) and the total genetic diversity (H_t) of the plant was recorded as 0.1120 and 0.3697 respectively. The observed genetic differentiation among populations (G_{st}) was 0.6970 and the estimate of gene flow among populations was 0.2174. The unweighted pair-group method using arithmetic average method of dendrogram was constructed using MEGA software. The genetic identity and difference among the populations were displayed in different clusters. The superior genotype was selected based on high genetic variability with more individual percentage of polymorphism.

Keywords :- Genetic Diversity, Western Ghats, *Andrographis paniculata*, ISSR-PCR analysis.

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INTRODUCTION

The wealth of medicinal flora in India is very high compared to other countries. Many plants and plant parts were directly or indirectly used as medicine in Traditional Medicinal Systems of India from time immemorial. It is estimated that 70-80% of people worldwide rely on traditional herbal medicine to meet their primary health care needs. Globally, millions of people rely on medicinal plants not only for income generation and livelihood improvement [1]. Cancer is the second serious killing disease in the world. For the control of disease many methods of treatment followed worldwide, but the mortality rate increased day by day. The methods of treatment like chemotherapy sometimes have a number of undesired side effects. The plant based natural cancer treatment may reduce adverse side effects. *Andrographis paniculata* also used for the preparation of anti-cancer drugs in drug developing medicinal plant industries. The traditional medicinal plant *Andrographis paniculata* (family Acanthaceae) is one of the most popular medicinal herbs used for the treatment of various diseases like cancer, influenza, diabetes, hypertension, ulcer, etc. in the continents of Asia, America and

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Africa[2]. Modern pharmacological studies suggested that *Andrographis paniculata* had the anti-tumor and immunomodulatory effects *in vitro* and *in vivo* [3]. The secondary metabolites including diterpenes, lactones and flavonoids existing in *Andrographis paniculata* were responsible for its anti-cancer activities [2, 4].

An understanding of the patterns of genetic variation within and among populations of medicinal plants like anticancer drug yielding plants are essential for devising optimum genetic resource management strategies for their conservation, sustainable utilization and genetic improvements. A decline in genetic variation can undermine the ability of an organism to respond to natural selection and consequently limits its evolutionary potential. Small populations are often subject to the loss of alleles through genetic drift or random fluctuations in allele frequency [5]. Molecular techniques for studying inter and intra specific variation may shed light on the role of migration in the evolutionary dynamics of these plants, which are very important for selection of superior plants. Molecular markers are recently used in many plant breeding and crop improvement studies of Reddy *et al.* [6]. DNA-based molecular is more reliable and mainly used for the improvement of medicinal plant species because the genetic information is very unique and not influenced by any factors like age, physiological conditions and environmental factors of the plants [7]. The molecular marker technique efficiency is based on the amount of polymorphism it can detect in the given accessions [8]. ISSR marker is a PCR based method, in which DNA sequences amplified at a distance between two identical microsatellite repeat regions oriented in opposite direction [9]. It utilizes repeat-anchored primers to amplify DNA sequences between two inverted SSRs. In the present investigation the genetic variability among the different accessions of *Andrographis paniculata* from the Western Ghats of Tamil Nadu was carried out using ISSR markers.

MATERIALS AND METHODS

The plant material selected for the present investigation is *Andrographis paniculata* Nees. belonging to the family Acanthaceae, one of the potent anti-cancer medicinal plants. The healthy young plant leaves were collected from the following ten different accessions of Western Ghats of Tamil Nadu viz., Kothayar (AP1), Sivagiri (AP2), Dindigul (AP3), Palani (AP4), Pollachi (AP5), Kalakad (AP6), Mettupalayam (AP7), Sathuragiri (AP8), Sathyamangalam (AP9) and Theni (AP10). The collected plant materials were transferred to zip lock cover and kept in ice box till to reach the laboratory. The plant material was stored in deep freezer for the isolation of DNA and ISSR Analysis.

Isolation of Genomic DNA

The genomic DNA was extracted using the standard protocol of Doyle and Doyle [10] with slight modification. The quality and quantity of the DNA was checked with 1% agarose gel electrophoresis and UV-Visible spectrophotometer respectively and the isolated genomic DNA was stored in deep freezer for further studies.

ISSR PCR Analysis

The amplification of PCR mixture was done with 20 µl reaction volume has 2x DyNAzyme II PCR Master Mix 10 µl. Among twenty primers, the following ten primers UBC811 (GAG AGA GAG AGA GAG AC), UBC812 (GAG AGA GAG AGA GAG AA), UBC823 (TCT CTC TCT CTC TCT CC),UBC834(AGAGAG GA GAGAGAGYT), UBC851 (GTG TGT GTG TGT GTG TYG), UBC855 (ACA CACACA CAC ACA CYT), UBC856 (AC CACACA CAC ACA CYA), UBC861 (ACC ACC ACC ACC ACC ACC), UBC881 (GGG TGG GGT GGG GTG) and UBC895 (AGA GTT GGT AGC TCT TGA TC) were selected for final experiment based on the ability of producing reproducible bands. The PCR reaction mixture was mixed gently in spinwin and 35 cycles of reaction was performed in a PCR (Applied Bio systems) with the amplification profile; of 95°C - 5.00 min for initial denaturation, 94°C -0.45 min for denaturation, 42°C - 1.00 min for annealing, 72°C - 1.30 min for extension, 72°C - 10.00 min for final extension and 4°C -∞ followed by cooling at 4°C. The final product of the PCR was checked in more than one percent agarose gels. The resulting gel was visualized and documented in Bio-Rad Gel documentation system.

Interpretation of Data

The presence and absence of clear, dense and visible bands in the gels of different populations were noted [11]. The data were analysed with the help of Pop gene package version 1.31and the dendrogram was constructed with MEGA software. Nei and Li[12] method was used for the calculation of individual population similarity index.

RESULTS AND DISCUSSION

In the present investigation the genetic diversity of anti-cancer drug yielding plant *Andrographis paniculata* was carried out. The plant materials were collected from ten different accessions of Western Ghats of Tamil Nadu. The DNA was isolated from young leaves of the selected plants and the ISSR- PCR analysis was carried out using ISSR primers. The data was analysed using the software PopGene and the dendrogram was constructed using MEGA software.

After screening twenty ISSR primers, 10 primers that displayed intense and reproducible bands were selected for further PCR amplification of *Andrographis paniculata*. ISSR markers amplified products produced a total of 50 loci in ten primers at ten different populations of *Andrographis paniculata* and generating high level of genetic polymorphism (Figure 1). The same type of bands occurred at different frequencies in all populations. The total numbers of bands produced in each primer were displayed in figure 2 and total number bands produced by each population were displayed in figure3. High genetic variation at species level was observed in the present investigation with the recording of percentage of polymorphic bands (PPB) at 96% with the number of polymorphic loci 48. The mean value of overall observed and effective number of alleles are 1.96 and 1.6435. Nei's gene diversity and Shannon information index is 0.3697 and 0.5446.

However, the genetic differentiation at population level was relatively low as compared to genetic variation evidenced at species level. This was proved by moderate PPB (%) recorded in the range of 14% to 44% averaging at 27.2%. Similarly, the effective number of alleles (N_e) varied from 1.1019 to 1.3249 with an average value of 1.2015, while the observed number of alleles (N_a) ranged from 1.14 to 1.44 averaging at 1.272. Nei's genetic diversity (*H*) also extended from 0.057 to 0.1808 with an average of 0.1120, and Shannon's information index (*I*) spanned from 0.0831 to 0.2629 with an average value of 0.1628. Among the different accession AP 4 (Palani) exhibited highest genetic diversity at population level while the lowest variation was found in AP 6 (Kalakad) (Table 1). Nei's unbiased genetic distance and identity was displayed in Table 2. Genetic distance between the populations was ranged from 0.2057 to 0.7713 and the genetic identity ranged from 0.5378 to 0.814.

Table 1: Genetic diversity within ten different populations of *Andrographis paniculata*

S. No	Accessions	N_a		N_e		H		I		NPL	PPL (%)
		Mean	SD	Mean	SD	Mean	SD	Mean	SD		
1	**AP1**	1.1800	0.3881	1.1296	0.3009	0.0728	0.1620	0.1062	0.2332	9	18
2	**AP2**	1.3600	0.4849	1.2591	0.3832	0.1456	0.2041	0.2125	0.2928	18	36
3	**AP3**	1.3200	0.4712	1.2000	0.3284	0.1185	0.1821	0.1771	0.2668	16	32
4	**AP4**	1.4400	0.5014	1.3249	0.4087	0.1808	0.2152	0.2629	0.3071	22	44
5	**AP5**	1.1600	0.3703	1.1315	0.3180	0.0706	0.1666	0.1008	0.2360	6	16
6	**AP6**	1.1400	0.3505	1.1019	0.2735	0.0570	0.1470	0.0831	0.2115	7	14
7	**AP7**	1.2000	0.4041	1.1591	0.3394	0.0863	0.1787	0.1240	0.2540	10	20
8	**AP8**	1.2600	0.4431	1.2058	0.3723	0.1118	0.1958	0.1607	0.2782	13	26
9	**AP9**	1.3600	0.4849	1.2696	0.3949	0.1493	0.2087	0.2166	0.2979	18	36
10	**AP10**	1.3000	0.4629	1.2334	0.3854	0.1276	0.2029	0.1839	0.2887	15	30
Average		1.272	0.4361	1.2015	0.3505	0.1120	0.3387	0.1628	0.2666	13.4	27.2
Over all		1.9600	0.1979	1.6435	0.2891	0.3697	0.1297	0.5446	0.1657	48	96.00

N_a, observed number of alleles; N_e, effective number of alleles; *H*, Nei's gene diversity; *I*, Shannon's information indices; NPL- Number of Polymorphic Loci PPL, percentage of polymorphicLoci; SD- Standard Deviation

Table 2: Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) of *Andrographis paniculata.*

Accessions	AP1	AP2	AP3	AP4	AP5	AP6	AP7	AP8	AP9	AP10
AP1	****	0.7942	0.7226	0.6391	0.6105	0.6418	0.6103	0.5703	0.5669	0.4624
AP2	0.2304	****	0.6933	0.7071	0.6228	0.6825	0.7054	0.6498	0.6769	0.5430
AP3	0.3248	0.3663	****	0.7448	0.6904	0.7557	0.7418	0.5378	0.6410	0.6043
AP4	0.4476	0.3466	0.2947	****	0.7054	0.7334	0.7935	0.6792	0.7452	0.6673
AP5	0.4935	0.4736	0.3705	0.3490	****	0.8077	0.7410	0.7082	0.6847	0.6283
AP6	0.4434	0.3820	0.2801	0.3101	0.2136	****	0.7647	0.6631	0.6921	0.6347
AP7	0.4938	0.3490	0.2987	0.2313	0.2998	0.2683	****	0.7062	0.7510	0.6415
AP8	0.5615	0.4311	0.6202	0.3869	0.3451	0.4108	0.3478	****	0.8141	0.6424
AP9	0.5675	0.3903	0.4447	0.2941	0.3787	0.3681	0.2863	0.2057	****	0.6940
AP10	0.7713	0.6107	0.5037	0.4045	0.4647	0.4547	0.4440	0.4426	0.3652	****

Table 3: Genetic diversity and differentiation of different populations of *Andrographis paniculata*

S.No	Primers	No. of polymorphic fragments	Total diversity (H_t)	Genetic diversity within population (H_s)	Genetic differentiation (G_{st})	Gene flow (N_m)
1	UBC834	5	0.2749	0.0592	0.6212	0.1197
2	UBC811	8	0.3869	0.1222	0.6810	0.2853
3	UBC812	7	0.4903	0.1635	0.6665	0.2542
4	UBC823	4	0.3394	0.1080	0.6437	0.2928
5	UBC851	3	0.3120	0.0909	0.6913	0.2535
6	UBC855	5	0.3932	0.1594	0.5850	0.3550
7	UBC856	6	0.4183	0.1546	0.6176	0.3518
8	UBC861	5	0.2994	0.0738	0.7260	0.2067
9	UBC881	4	0.3480	0.0882	0.5595	0.1363
10	UBC895	5	0.4302	0.1042	0.7430	0.1844
Average		**9.45**	**0.36926**	**0.1124**	**0.65348**	**0.2450**
Overall population & Mean value			**0.3697**	**0.1120**	**0.6970**	**0.2174**

H_t, total genetic diversity; H_s, genetic diversity within populations; G_{st}, the relative magnitude of genetic differentiation among populations; N_m, estimate of gene flow among populations.

Figure 1: ISSR- PCR profiles of *Andrographis paniculata*

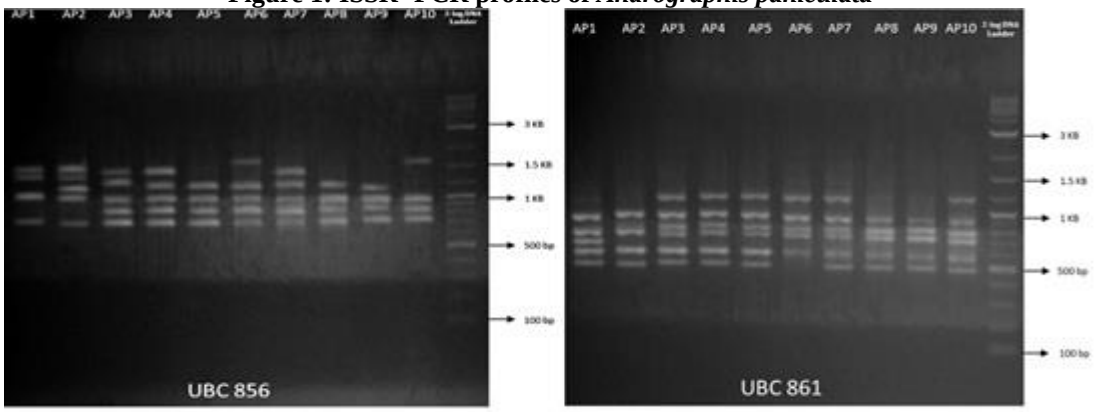


Figure 2: Total Number of bands produced by each primer in *Andrographis paniculata*

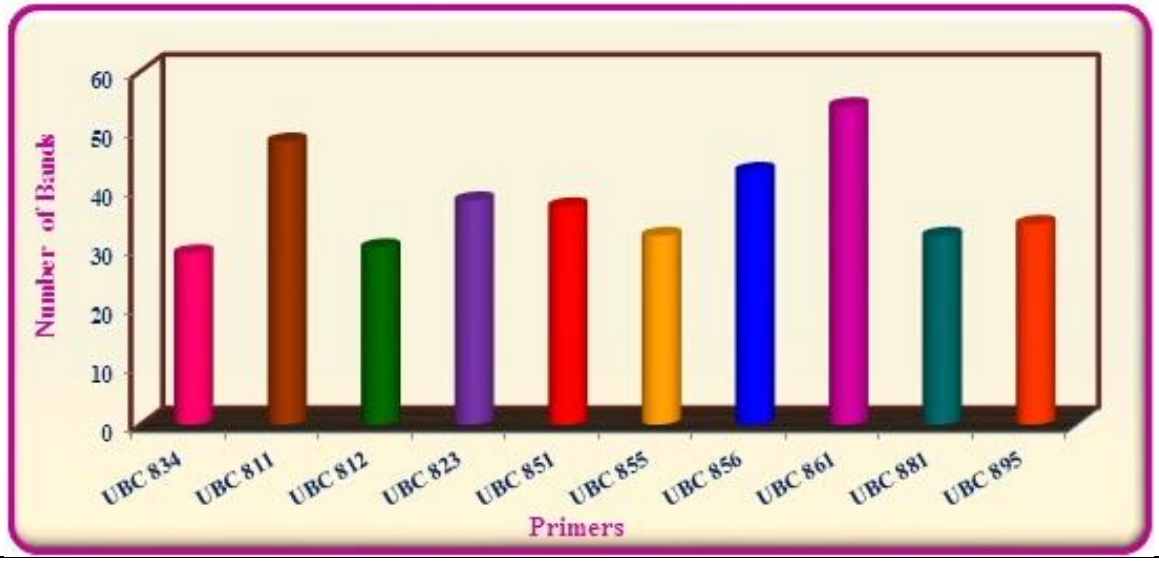


Figure 3: Total Number of bands produced in each population of *Andrographis paniculata*

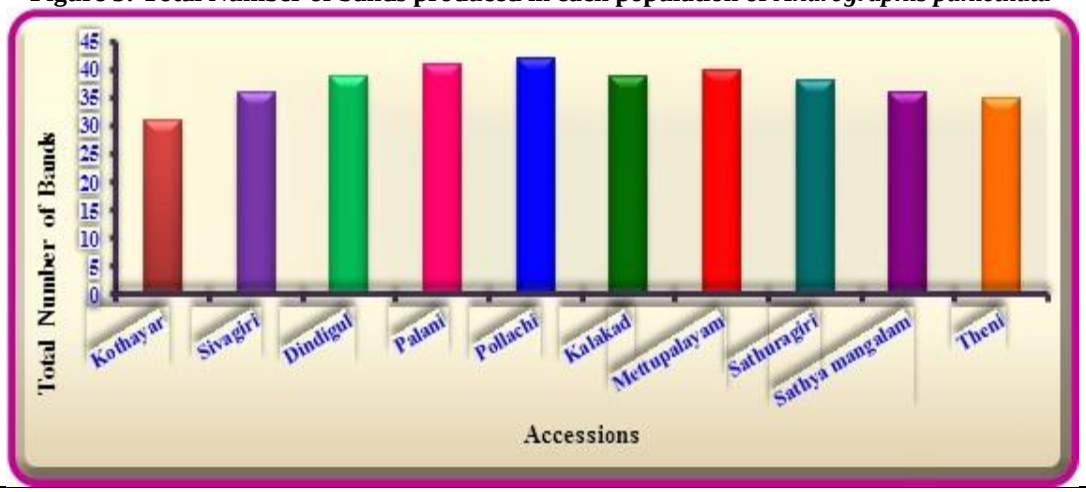
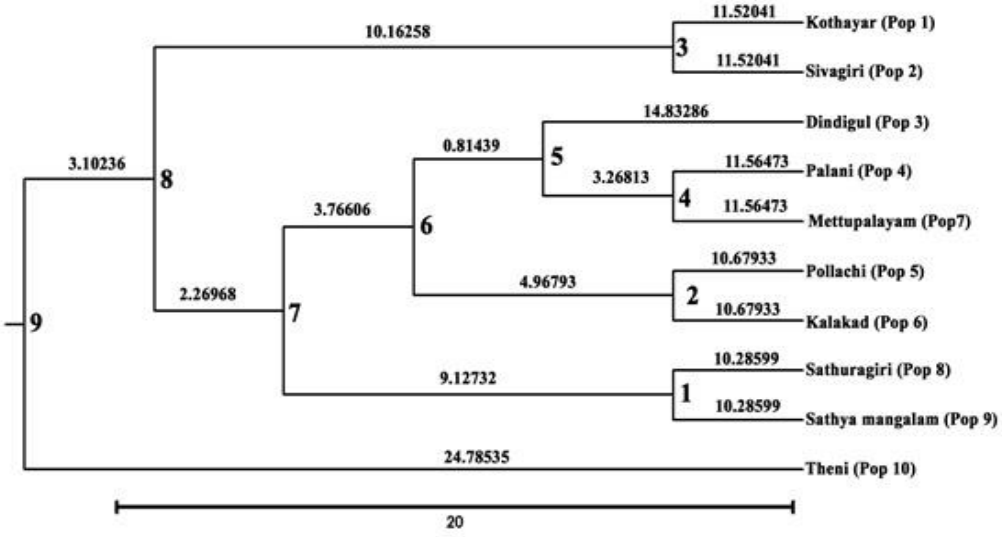


Figure 4: UPGMA Dendrogram showed the distance between the different accessions *Andrographis paniculata* using ISSR Markers.



The average gene diversity within the population (H_s) was 0.1124, the highest (H_s) was 0.1635 and the lowest (H_s) was 0.0592. The total diversity (Ht) ranged from 0.2749 to 0. 0.4903 and the average was 0.1124. The average level of genetic differentiation (G_{st}) between the populations was 0.65348 and the G_{st} ranged from 0.3252 to 0.6298. The average estimated gene flow from one population to the other generation (N_m) was 0.2450; while the lowest was 0.5595 and the highest was 0.7430 between the populations. The mean value of genetic diversity within population (H_s) and the total genetic diversity (H_t) of the plant were recorded at 0.1120and 0.3697 respectively. The observed genetic differentiation among populations (G_{st}) was 0.6970 and the estimate of gene flow among populations was 0.2174 (Table 3).

The similarity matrix obtained in the present study was used to construct a dendrogram with the UPGMA method and resulted in their distant clustering in the dendrogram. The length between populations mentioned in the branches of dendrogram (Figure 4). The dendrogram of *Andrographis paniculata* showed distinct separation of the ten accessions with five major clusters and four minor clusters. The first cluster occurs in between Pop 8 (Sathuragiri) and Pop9 (Sathyamangalm), second cluster occurs in between Pop5 (Pollachi) and Pop6 (Kalakad). Third and fourth clusters occurs inbetween Pop 1 (Kothayar), Pop2 (Sivagiri) and pop 4 (Palani), Pop7 (Mettupalayam) respectively. In the dendrogram Pop 3 (Dindigul) and Pop 10 (Theni) coming under separate clusters with more distance. Due to the anthropogenic pressure on collection of medicinal plants in wild condition leads to the problem in their population, studies that address the analysis of genetic diversity are fundamental to select priority genotypes that may serve to guide future pharmacological studies [14]. Genetic

diversity of plants of the same species can result in the production of several active compounds, and consequently in several biological properties since genetic factors can influence the synthesis of these compounds. Molecular markers will help to find out the genetic relationship between the individuals of the same species and clustering the genetically similar ones. Molecular markers are the efficient and extremely appropriate tools for the elaboration of conservation strategies of medicinal plants, as well as for the use of plant resources in future breeding
programs.

CONCLUSION

The superior genotypes of *Andrographis paniculata* was identified from different accessions where the plant was collected based on percentage of individual polymorphism within and between the populations. High genetic variability with more individual percentage of polymorphism specifies the superior genotype. Among the studied ten populations of *Andrographis paniculata*, Pop 4 (Ap-4 - Palani) showed more percentage of polymorphic loci compared to other accessions. So the present study concluded that Palani population will be the superior genotype of studied areas of Western Ghats of Tamil Nadu for *Andrographis paniculata*.

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