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Original Article

RAPD Analysis of Genetic Variability in Wild Populations of *Withania somnifera* (L.) Dunal

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Abstract

Withania somnifera is extensively used as herbal medicine, because it contains clinically important compounds. In the present study, the genetic variability in *W. somnifera* among accessions of different geographical region in Tamil Nadu was assessed through Random Amplified Polymorphic DNA (RAPD) markers. Five accessions of *W. somnifera* were screened with ten primers of which six primers were found to be the most informative. These primers produced multiple band profiles with a number of amplified DNA fragments varying from 5 to 9. A total of 37 polymorphic bands were observed. The genetic distance between the population ranged from 0.2436 to 0.4754 and the genetic identity ranged from 0.6216 to 0.7838. The overall observed and effective number of alleles was about 1.6216 and 1.4271 respectively. Nei's over all genetic diversity is 0.2465. The degree of percentage of polymorphism (83.78 %) was found to be high in accession collected from Samykoundanpalayam in Tirupur District, Tamil Nadu. The information obtained here could be valuable for devising strategies for conservation this medicinal plant.
Keywords: Genetic variability, RAPD-PCR analysis, *Withania somnifera* (L.) Dunal

Introduction

Biodiversity can be defined at genetic, specific and community levels of biological organization and its variability among living organisms in all sources. The value of biodiversity is the difference between the current or future value of a diverse range of genus/species/ ecosystem and the value of a less-diverse range. It is not the gross value of all naturally derived goods and services. Plant diversity is an irreplaceable resource, providing raw materials for introduction, domestication as well as improvement programmers in agriculture and forestry (Kushwaha and Kumar, 1999).

Information on genetic diversity and relationship among and between individuals, populations, plant varieties, animal breeds and species are of importance to plant and animal breeders for the improvement of crop plants and animal breeds for conservation biology and for studying the evolutionary ecology of populations. Genetic diversity studies can identify alleles that might affect the ability of the organism to survive in its existing habitat, or might enable it to survive in more diverse habitats. This knowledge is valuable for germplasm conservation, individual, population, variety or breed identification (Duran *et al.*, 2009). Various types of markers such as morphological, chromosomal, biochemical and molecular markers are used for this purpose (Anurag *et al.*, 2008).

Various types of molecular markers are utilized to evaluate DNA polymorphism. Random amplified polymorphic DNA (RAPD), a PCR based technique is simple, cost-effective and a powerful tool in the analysis of plant genome characterization. Although, RAPD is criticized for its low reproducibility (Hansen *et al.*, 1998, Virk *et al.*, 2000), it is overcome by optimization of the RAPD reaction and maintenance of stringent conditions. RAPD has, therefore, been extensively used in assessing genetic diversity and relationship measures in various plant species (Barker et al., 1999, Bauvet *et al.*, 2004; Upadhyay *et al.*, 2004).

Withania somifera (L.) Dunal is an important tropical medicinal plant belongs to the family Solanaceae. Local name: Ashwagandh, Amukara (Yang *et al.*, 2007). It is widely claimed to have potent aphrodisiac, sedative, rejuvenative and life prolonging properties. It is also used as a general energy-enhancing tonic known as Medharasayana, which means 'that which promotes learning and a good memory' and for geriatric problems (Williamson, 2002). The leaves of the plant are bitter in taste and used as an antihelmantic. The infusion is given in fever. Bruised leaves and fruits are locally

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applied to tumors and tubercular glands, carbuncles and ulcers (Kapoor, 2001). The roots are also used in constipation, senile debility, rheumatism, general debility, nervous exhaustion, loss of memory, loss of muscular energy and spermatorrhoea (Watt, 1972).

In the present study, analysis of genetic diversity of *Withania somnifera* collected from different locations of Tamilnadu has been carried out using RAPD technique.

Materials and Methods

The samples of *Withania somnifera* were collected from five locations of Peikulam (Thoothukudi Dist.), Samykoundan Palayam (Tirupur Dist.), Thirupasethi (Sivagangai Dist.), Thiruthalaiur (Trichy Dist.) and Thenkanikotta (Krishnagiri Dist.) in Tamil Nadu. The collected plant materials were transferred to plastic bags for transport from field to laboratory and permanently stored at -70^{o} C. DNA was isolated (Doyle and Doyle, 1990) and then RAPD analysis (Williams *et al*., 1990) was carried out.

The amount of DNA and its quality were assessed by UV spectrophotometer. The DNA is pure enough (OD260/280 =1.68), (Sambrook and Russel, 2000) for RAPD- PCR analysis. RAPD amplification was performed in thin-walled micro centrifuge tubes using a thermocycler. Reaction volume of 25 µl was composed of 2.5 µl of 10 X PCR buffer, 0.3 µl of $MgCl_2$, 4.0 µl of dNTPs, 2.4 µl of primer, 0.5 µl of Taq polymerase, 13. 3 µl of sterile dis. H_20, 2.4 µl of primer and 2 µl of DNA template. The amplification program was as follows: 5 min at 94°C for initial denaturation, (1 min at 94°C for denaturation, 1 min at 37 °C for annealing, 1 min at72 °C for extension- 35 cycles), 5 min at 72 °C for final extension and finally stored at 4 °C forever.

Earlier, ten primers had been tested and six primers which produced reproducible bands were selected. The experiments were repeated three times and confirmed the reproducibility of bands. The amplification products were separated by electrophoresis in agarose gel (1.4%). The gel was visualized by UV transilluminator and photographed with gel documentation system Alpha Imager 1200. Based on the primary data (presence or absence of bands), pair wise genetic distance between the samples were calculated using POPGENE package version 1.31 (Yeh *et al*., 1999).

Results and Discussion

Genetic variability in *Withania somifera* in different locations Tamil Nadu has been carried out using RAPD markers. Six primers generated reproducible, informative and easily scorable RAPD profiles (Table 1 & Plate 1).

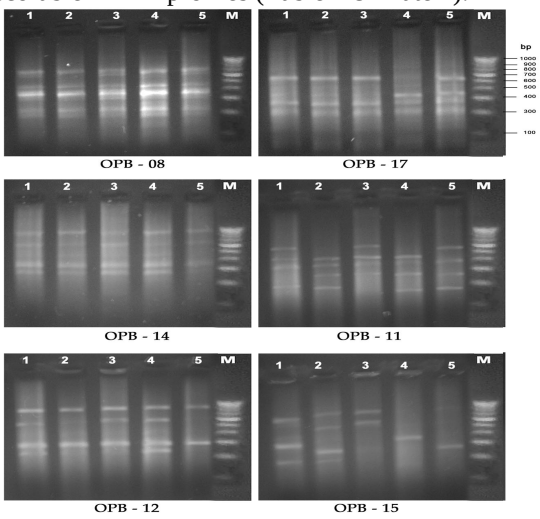

Plate-1: RAPD banding pattern of *W. somnifera*

These primers produced multiple band profiles with a number of amplified DNA fragments varying from 5 to 9. A total of thirty seven polymorphic bands were observed. The same type of bands occurred at different frequencies in all populations. There were many additional bands neglected which were not reproducible.

Table- 1: Genetic and gene diversity within and between the populations of *W. somnifera* for RAPD markers

S.No	Primers	Sequence 5'-3'	No. of polymorphic fragments	H_T	*Hs*	G_{ST}	*Nm*	Band Frequency
1.	OPB08	GTCCACACGG	9	0.4755	0.4027	0.1534	2.7594	0.7326
2	OPB17	AGGGAACGAG	6	0.3685	0.3374	0.1518	2.7938	0.7322
3	OPB14	TCCGCTCTGG	5	0.3598	0.3022	0.2168	1.8062	0.7333
4	OPB11	GTAGACCCGT	6	0.4441	0.3835	0.1524	2.7808	0.7267
5	OPB12	CCTTGACGCA	5	0.4807	0.4090	0.1455	2.9364	0.7311
6	OPB18	GGAGGGTGTT	6	0.4227	0.3992	0.0877	5.2012	0.7338

H_T - Total diversity; *Hs* – Gene diversity within populations; G_{ST} - Genetic differentiation; *Nm* – Gene flow

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Genetic and gene diversity measures were calculated according to Nei's index using POPGENE software and results were depicted in the Table 1. The mean genetic heterozygosity or diversity (*H*) ranged from 0.2911 to 0.3929. Nei's (1978) overall genetic diversity is 0.2465. The genetic distance between the population ranged from 0.2436 to 0.4754 and the genetic identity ranged from 0.6216 to 0.7838 which is shown in Table 2. The overall observed and effective number of alleles was about 1.6216 and 1.4271 respectively. The highest percentage of polymorphism was 83.78 among the populations (Table 1). The highest *Hs* was 0.4090 and the lowest *Hs* was 0.3022. The total diversity (H_T) ranged from 0. 3598 to 0.4807. The mean genetic differentiation (G_{ST}) between populations over all loci was 0.37 and the G_{ST} ranged from 0.0877 to 0.2168. The band frequency ranged from 0.7267 to 0.7338. The average gene flow from one population to the other generation (*Nm*) was 3.0463; The observed number of alleles (*Na*), the effective number of alleles (*Ne*) Shannon Information Index (*I*) and number of polymorphic loci (NPL) for all the accessions of *W. somnifera* are depicted in the Table 3.

Table- 2: Analysis of polymorphism obtained with RAPD primers in different accessions of *W. somnifera*

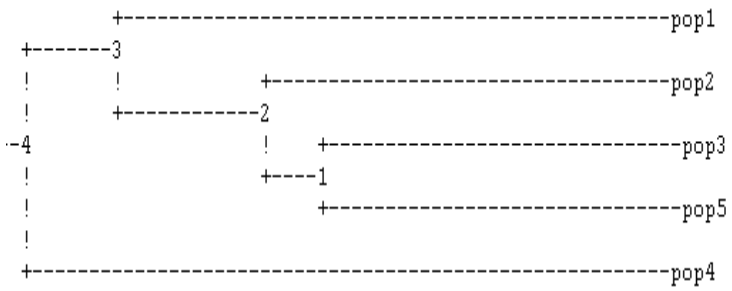
Accession	*Na*	*Ne*	*H*	*I*	NPL	% of polymorphism
Pop 1	1.7297	1.6396	0.3368	0.4762	27	72.97
Pop 2	1.8378	1.7482	0.3929	0.5539	31	83.78
Pop 3	1.6486	1.5473	0.2911	0.4142	24	64.86
Pop 4	1.7838	1.7103	0.3716	0.5226	29	78.38
Pop 5	1.7297	1.6388	0.3358	0.4749	27	72.97

Na – Observed number of alleles; *Ne* – Effective number of alleles; *H* – Gene diversity; *I* – Shannon Information Index; NPL – Number of Polymorphic Loci; Pop 1- Peikulam; Pop 2- Samykoundan Palayam; Pop 3- Thirupasethi; Pop 4- Thiruthalaiur; Pop 5- Thenkanikotta

Table 3. Nei's unbiased measures of Genetic distance and Genetic identity for *W. somnifera*

Pop ID	Peikulam	Samykoundan Palayam	Thirupasethi	Thiruthalaiur	Thenkanikotta
Peikulam	****	0.7027	0.7297	0.6757	0.6216
Samykoundan Palayam	0.3528	****	0.7568	0.6486	0.7568
Thirupasethi	0.3151	0.2787	****	0.6216	0.7838
Thiruthalaiur	0.3920	0.4329	0.4754	****	0.6216
Thenkanikotta	0.4754	0.2787	0.2436	0.4754	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal) Pop 1- Peikulam, Pop 2- Samykoundan Palayam, Pop 3- Thirupasethi, Pop 4- Thiruthalaiur and Pop 5- Thenkanikotta



Pop 1- Peikulam, Pop 2- Samykoundan Palayam, Pop 3- Thirupasethi, Pop 4- Thiruthalaiur and Pop 5- Thenkanikotta

Figure-1: Dendrogram based on Nei's Genetic distance

Genetic variation in a population is measured by the heterozygosity or the degree of polymorphism. For the conservation of a species, genetic variability is of the utmost importance.

In order to study the correlation between populations, UPGMA algorithm was used to predict a dendrogram for the five populations of *W. somnifera* (Fig 1) using popgene soft ware. It showed distinct separation of the collected accessions from five locations into two major clusters. Pop 4 formed a separate clade where as Pop 1, 2, 3 and 5 diverged from Pop4. From this

we can conclude that the Pop4 is more diversified. In the dendrogram based on Nei's genetic distance obtained (Fig 1), the populations were highly differentiated by their own genetic distance.

The clustering results of different accessions suggest that *W. somnifera* L. undergoes major part of genetic variation by environmental factors. Genetic diversity refers to the variation at the level of individual genes (polymorphisms), and provides a mechanism for populations to adapt to their ever-changing environment. The genetic variability in *W. somnifera* may be partly explained as a result of abiotic and biotic factors.

It is desired to maximize the preservation of alleles. Geographical, climatic or reproductive variables explain the partitioning of the diversity observed which may aid in improving the strategies for maximizing the efficiency of germplasm collection and preservation.

The utility of RAPD markers in estimating genetic variability has been demonstrated in several studies. A similar study was done in *Withania somnifera* by Bilal *et al.* (2010). 7 populations of *W. coagulans* from the districts of Kohat and Karak in Pakistan were analyzed by Syed (2009). Finger *et al.* (2010) estimated the genetic diversity of 49 accessions of *Capsicum chinensis.* Molecular analysis in *Urginea indica* collected from different location of Karnataka was reported by Harini *et al.* (2008). Ruan *et al.* (2008) analyzed the DNA molecular characters of *Centella asiatica* with RAPD technology. Rout (2006) reported the genetic variation within 15 clones of *Tinospora cordifolia* through RAPD markers. Genetic diversity analysis in *Rauvolfia serpentina* and *Rauvolfia tetraphylla* L, using RAPD Markers was carried out by Padmalatha and Prasad (2006); Padmalatha and Prasad (2007).

Conclusion

Analysis of RAPD could be useful to detect genetic differentiation of *Withania somnifera* among five locations in Tamilnadu. From the work done in this medicinal plant collected at different geographical locations, it was understood that each location varied with respect to environmental factors and genetic parameters. Thus by analyzing the genetic variability we are able to identify the elite population. The degree of percentage of polymorphism was found to be high (83.78 %) in accession collected from Samykoundan Palayam of Tirupur District of Tamilnadu and was concluded to be the superior genotype. The information obtained here could be valuable for devising strategies for conservation of *W. somnifera*.

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