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Analysis of Genetic Variation among Populations of *Withania somnifera* (L.) in South India based on RAPD Markers

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Authors' contributions

This work was carried out in international collaboration between all authors. Author RU performed the experiment and the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SK, TSM and BS assisted the collection of samples and the technical experiment. Author AG designed the study and managed the analyses of the study. Authors EJK and KMJ supported the experiment technically and managed the literature searches. Authors CWC and SCK performed the data analysis, wrote the final version of the manuscript and funded a grant from Korean Government. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: The present study was carried out to analyze the genetic variations among 20 different populations of *Withania somnifera* (L.) Dunal collected from different habitats (locations) by RAPD analysis.
Methodology: DNA was isolated from the fresh leaf samples collected from the field by Bernatsky and Tankley method. Isolated genomic DNA was purified by phenol: chloroform: isoamyl alcohol (25:24:1) extraction mixture and then amplified by MJ themal cycler. Amplified DNA products were quantified and then subjected to RAPD analysis by the method of Williams et al.
Results: Randomly amplified polymorphic DNA (RAPD) was used to analyze the genetic

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variation and relationship among 20 populations of *Withania somnifera* collected from different part of South India, including the states of Tamilnadu, Puducherry, Kerala, Andhra Pradesh, Karnataka, Gujarat, Maharashtra and supplemented by two commercial varieties from Uttar Pradesh and Delhi. Out of 40 primers, 11 selected primers produced 96 consistent RAPD markers ranging in size from 0.2 kb to 4.0 kb; out of which 75 were polymorphic. Similarity indices were estimated using the Dice coefficient of similarity and cluster analyses were carried out on the similarity estimates using the unweighted pair-group method to produce a dendrogram using arithmetic average (UPGMA) in the NTSYSpc-verson 1.80 software. The similarity coefficient ranges from 0.53 to 0.98, suggesting that the pronounced genetic variations exist among populations of *W. somnifera* in South India. The cluster analysis indicates that the 20 populations of *W. somnifera* were divided into five major groups, regardless of geographical locations.
Conclusion: The RAPD analysis indicates existence of genetic variations in natural populations and it may influence and produce changes in phytochemical constituents of *W. somnifera* populations.

Keywords: Withania somnifera; RAPD; genetic variation; similarity; populations; geographical locations.

1. INTRODUCTION

Withania somnifera (L.) Dunal, most commonly known as ashwagandha, is a perennial plant belonging to the Solanaceae. Ashwagandha roots are compared with ginseng roots for their restorative properties and have been given the name 'Indian ginseng'. The entire plant of *W. somnifera* is used for the treatment of tuberculosis, rheumatism, inflammatory conditions, and cardiac diseases [1]. In addition, it is used as an antitumor, antibiotic, anticonvulsant and CNS-depressant agent [2]. The pharmacological activities of *Withania somnifera* like reverses Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver [3] inhibits amyloid beta fibril formation *in vitro* [4] and propoxur-induced acetylcholine esterase inhibition and impairment of cognitive function attenuation [5] were recently reported.Other traditional uses and biological activities of *W. somnifera* are well described [6]. In previous studies, we observed the hypoglycaemic and hypolipidaemic effects of *W. somnifera* root and leaf extracts on alloxan-induced diabetic rats [7] and suggested that the presence of phenolic compounds including flavonoids in *W. somnifera* root and leaf extracts and their antioxidant activity may play a vital role in reduction of blood glucose level in alloxan-induced diabetic rats [8]. There are a number of reports elucidating the chemical and pharmacological properties of *W. somnifera* [9,10]. Recently researchers were also reviewed phytochemical and pharmacological profile of *Withania somnifera* [11,12,13]. The major constituents of *W. somnifera* are withanone and withanolides [9,10]. The presence of catechin was reported in *Withania somnifera* by high performance liquid chromatography analysis [14]. Qualitative estimation of *Withania somnifera* in polyherbal formulation by HPTLC method was documented recently [15].

The geographic distribution of *W. somnifera* extends widely from the Atlantic Ocean to the South - East Asia and from the Mediterranean region to South Africa and India [16]. The cultivation of *W. somnifera* is spread over about 4000 hectares throughout India. Both wild and cultivated populations of this species exhibit enormous diversity in chemical constituents. However, there is an apparent lack of improved varieties of *W. somnifera* to make its cultivation cost-effective and pharmacologically valid. Studies pertaining to the identification

of morphological and physiological variations based on extremely diversified geographical distribution of *W. somnifera* have been conducted by [17]. An extreme degree of variability was also recorded in *W. somnifera* with respect to growth habit and morphological characteristics of plants in different parts of India and in other countries [18]. As Indian natural population exhibits inheritable variations, its documentation and molecular confirmation can help to evolve promising high yielding cultivar. In addition, this will serve us genetic stock for future breeding programs.

Molecular markers generally refer to biochemical constituents, including primary and secondary metabolites and other macromolecules such as nucleic acids. Secondary metabolites as markers have been extensively used in quality control and standardization of botanical drugs. It has been well documented that geographical conditions affect the active constituents of the medicinal plant and also their activity profiles [19]. In our previous study revealed that the *W. somnifera* leaf and root extracts from five geographical locations possess different antioxidant capacities due to various amounts of phenolic compounds [20]. However, it is often difficult to accurately discriminate species within the same genus based on these subjective markers. Besides, the use of chromatographic techniques and marker compounds to standardize herbal medicines is also limited because of variable chemical complexity, which is affected by growth, storage conditions, harvest times, and variable sources [19,21].

DNA markers are considered as reliable sources to indicate the existence of polymorphism and unique genetic composition of chemotypes which is not affected by age, physiological conditions or environmental factors [22]. Random amplified polymorphic DNA (RAPD) has the advantage of being quick and easy, requiring little plant material, and having a high resolution [23,24], although it has some disadvantages [25,26]. The RAPD analysis has been applied in herbal medicine to discriminate between species in various genera [26]. Genetic variation within and between *Withania* species by amplified fragment length polymorphism (AFLP) has been studied by [18]. Comparative analysis of the efficiency of selectively amplified microsatellite polymorphic loci (SAMPL) and AFLPs in assessing genetic relationships among *W. somnifera* genotypes was also reported by [27]. In addition, phytochemical and genetic relationships in selected chemotypes of *W. somnifera* were analyzed using AFLP markers [28]. These reports concentrate only on genetic diversity analysis of North Indian populations, but till date no information was available on South Indian *W. somnifera* populations.

Therefore, the present study aimed to 1) characterize the morphological variation by plant characteristics and the genetic variation through RAPD analysis among *W. somnifera* populations collected from 18 locations in South India and 2 locations in North India, 2) construct a dendrogram for the clustering analysis among *W. somnifera* populations, and 3) determine the relationship between the genetic variation and geographical locations.

2. MATERIALS AND METHODS

2.1 Plant Populations (Accessions)

Seeds of *W. somnifera* were collected from natural habitat and reputed medicinal plant Research stations of South India. Eighteen populations were collected from the states of South India including Tamilnadu, Puducherry, Kerala, Andhra Pradesh, Karnataka, Gujarat and Maharashtra and were supplemented by two North Indian commercial varieties from

Uttar Pradesh and Delhi (Fig. 1 and Table 1). Seeds of 10 Tamilnadu populations were collected from the different places with minimum 100 km distance; these included Pudukkottai (WS-01), Perambalur (WS-03), Karur (WS-04), Tanjore (WS-05), Periyakulam (WS-06), Coimbatore (WS-09), Cumbum (WS-11), Yercaud (WS-12), Madurai (WS-17), and Nagapattinam (WS-20). Tamilnadu is located in the south eastern part of Indian peninsula and its climate is tropical with a little variation in temperatures during winter and summer. Two North Indian commercial varieties collected from Lucknow in Uttar Pradesh (WS-02 in this study but originally WSL-10) and Delhi (WS-07 in this study but originally JS-20), were used as control varieties. The collected seeds were sowed in the field of experimental garden at the Department of Biotechnology, Bharathidasan University, India for uniform plantation.

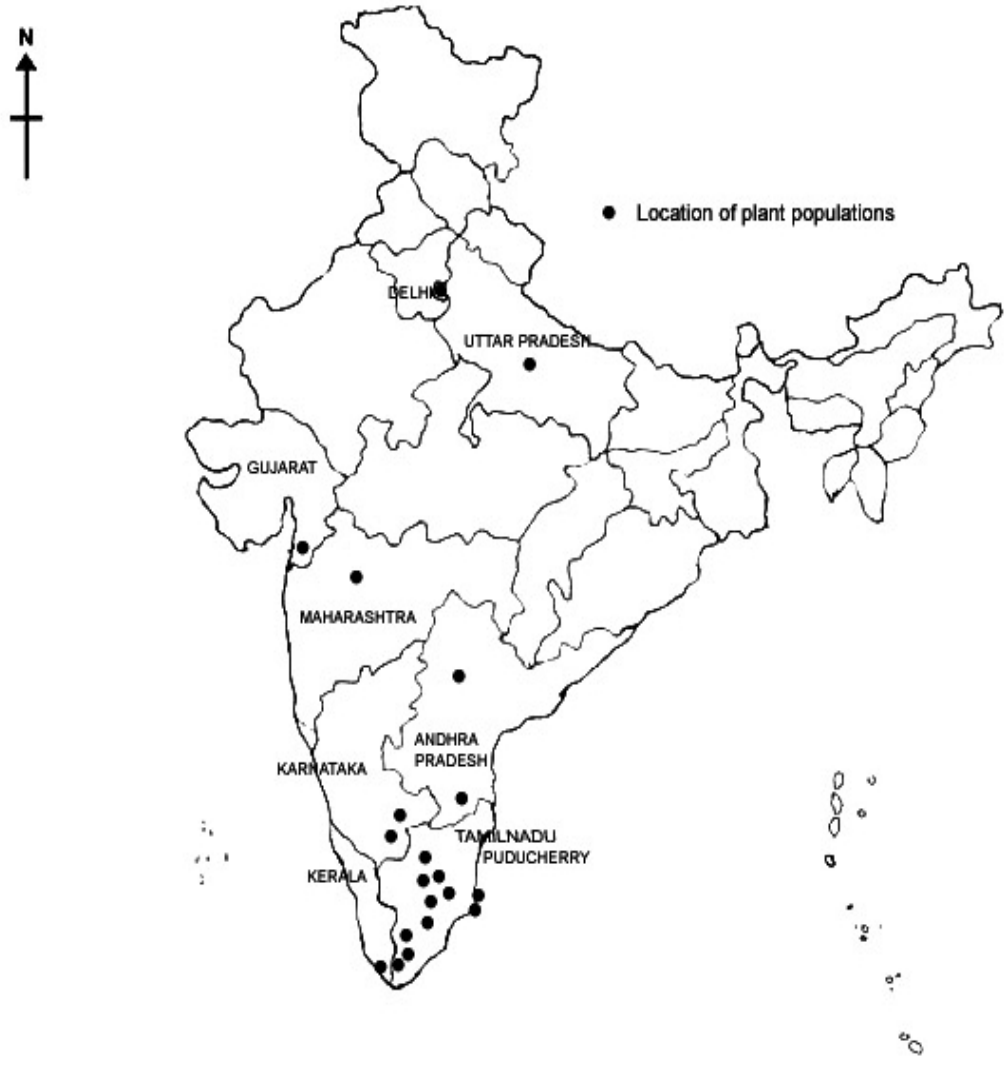

Fig. 1. Sampling locations of 20 different populations of *W. somnifera* (L.) Dunal

Table 1. Different populations of *W. somnifera* used as sources of DNA.

Populations/ Accession number	Locations	State	Longitude	Latitude	Temperature (ºC)
WS-01	Pudukkottai	Tamilnadu	78º 26' 50" E	09º 50' 45" N	41 (S) / 18 (W)
WS-02	Lucknow	Uttar Pradesh	80º 55' 00" E	26º 51' 00" N	47 (S) / -1 (W)
WS-03	Perambalur	Tamilnadu	78º 52' 59" E	11º 13' 59" N	42 (S) / 24 (W)
WS-04	Karur	Tamilnadu	78º 04' 59" E	10º 57' 00" N	39 (S) / 19 (W)
WS-05	Tanjore	Tamilnadu	79º 09' 00" E	10º 48' 00" N	36 (S) / 23 (W)
WS-06	Periyakulam	Tamilnadu	77º 33' 00" E	10º 07' 00" N	39 (S) / 16 (W)
WS-07	Delhi	Delhi	77º 13' 00" E	28º 40' 00" N	45 (S) / 05 (W)
WS-08	Aurangabad	Maharashtra	75º 23' 54" E	19º 53' 47" N	40 (S) / 05 (W)
WS-09	Coimbatore	Tamilnadu	76º 51' 58" E	10º 56' 20" N	34 (S) / 16 (W)
WS-10	Karaikal	Puducherry	79º 49' 48" E	11º 55' 48" N	40 (S) / 24 (W)
WS-11	Cumbum	Tamilnadu	77º 18' 00" E	09º 44' 00" N	35 (S) / 22 (W)
WS-12	Yercaud	Tamilnadu	78º 14' 00" E	11º 46' 00" N	30 (S) / 13 (W)
WS-13	Hyderabad	Andhra Pradesh	78º 27' 30" E	17º 25' 39" N	43 (S) / 23 (W)
WS-14	Valsad	Gujarat	72º 55' 59" E	20º 37' 59" N	42 (S) / 14 (W)
WS-15	Bangalore	Karnataka	77º 36' 01" E	13º 06' 26" N	36 (S) / 19 (W)
WS-16	Tirupathi	Andhra Pradesh	79º 25' 00" E	13º 39' 00" N	45 (S) /10 (W)
WS-17	Madurai	Tamilnadu	78º 03' 35" E	10º 01' 54" N	37 (S) / 21 (W)
WS-18	Trivandrum	Kerala	76º 55' 00" E	08º 29' 00" N	32 (S) / 21 (W)
WS-19	Mysore	Karnataka	76º 15' 36" E	12º 39' 21" N	35 (S) / 16 (W)
WS-20	Nagapattinam	Tamilnadu	79º 50' 79" E	10º 46' 47" N	41 (S) / 24 (W)

S : summer, W : winter

2.2 Morphological Characterization

Leaf characteristics [29] such as color, shape, tip and size and other characteristics were studied in accordance with National Bureau of Plant Genetic Resources (NBPGR), New Delhi, to compare the morphological similarity of populations.

2.3 DNA Extraction

Fresh leaf samples were collected from the field and used for the isolation of DNA [30]. About 2 grams of leaf tissue was frozen with liquid nitrogen and ground into a fine powder and then added 10 ml of preheated (65ºC) extraction buffer containing 1.5% (w/v) hexadecyl or cetyl trimethyl ammonium bromide (CTAB), 10 mM Tris HCl (pH 8.0), 1.4 M sodium chloride, 20 mM EDTA and 0.1% (v/v) 2-mercaptoethanol. The mixture was incubated in a water bath for 30 min at 65ºC. An equal volume of chloroform: isoamyl alcohol mixture (24:1, v/v) was added, then gently mixed for 15 min and centrifuged at 10,000 rpm for 20 min at room temperature. The aqueous phase was collected and an equal volume of ice-cold isopropanol was added and mixed gently until the DNA was precipitated out. The precipitated DNA was dissolved in 200-500 µl of TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0). The contaminant RNA was eliminated from DNA by treating with RNase to a final concentration 20 µg/ml. To the DNA sample, an equal volume of phenol: chloroform: isoamyl alcohol mixture was added and mixed well, then centrifuged at 10,000 rpm for 10 min at room temperature. The aqueous phase was collected and an equal volume of chloroform was added and mixed then it was centrifuged at 10,000 rpm for 2 min. To the collected aqueous phase $1/10^{th}$ volume of 3 M sodium acetate (pH 8.0) and 2 volumes of absolute ethanol was added and thoroughly mixed then kept at –20ºC for half an hour. DNA was pelleted by centrifugation at 10,000 rpm for 5 min. The resulting pellet was dissolved in nuclease free water after which the quality and quantity of the DNA was checked using spectrophotometer and agarose gel (1.5%) electrophoresis. The absorbance ratio of DNA sample between 260 and 280 nm was recorded and the quality of the genomic DNA was confirmed. Final DNA sample was stored at 4ºC for further RAPD analysis.

2.4 RAPD Protocol and Primer Screening

The RAPD analysis was carried out by the method of [31]. About 50 ng of DNA samples were taken in PCR tubes and mixed with 200 µM of each dNTPs, 0.5 µM RAPD primer (Operon Technologies, Alameda, California), 25 mM $MgCl_2$, 1 unit of *Taq* polymerase and reaction buffer (Genei, Bangalore, India). Finally the total reaction mixture volume was made up to 25 µl by nuclease free water. The reaction tubes were placed in an MJ thermal cycler (USA) and the amplification was performed with a temperature program consisting of the initial denaturation at 95ºC for 3 min followed by 35 cycles of denaturation at 94ºC for 1 min, annealing for 2 min at 37ºC and polymerization at 72ºC for 3 min. Final extension was at 72ºC for 7 min. After the completion of PCR amplification, the products were analyzed by electrophoresis using 1.5% agarose gel stained with 0.5 µg/ml ethidium bromide. After electrophoresis the gels were documented in the gel documentation system (Vilber Lourmat, France). The analysis was performed for all the samples at least three times with each selected primer. The amplification products size was calculated by using the software photocapt MW. Forty 10-mer primers, corresponding to kits A and B from Operon Technologies (Alameda, California) were initially screened using different populations of *W. somnifera* to determine the suitability of each primer for the study. Eleven primers were selected for further analysis based on their ability to detect distinct, clearly resolved and

polymorphic amplified products of the populations of *W. somnifera.* All RAPD reactions were carried out with the same cycling conditions and chemicals. Fragment sizes of the amplification products obtained using RAPD primers were estimated from the gel by comparison with standard molecular weight marker (DNA ladder from Genei, Bangalore).

2.5 Data Analysis

PCR products from individual plants were scored as either present or absent. Only clearly amplified fragments were analyzed. Scores of 1 (present) or 0 (absent) were used to form a matrix. The genetic distance was calculated as the percentage of the total number of bands scored that were clearly different between each pair of populations. Each amplification fragment was named by the source of the primer (Operon Technologies, Alameda, California) kit letter or number, and its approximate size in base pairs. The samples were considered to be similar or identical if there was a complete concordance of DNA fragment profiles and considered different if there was a difference of one or more DNA bands. Similarity indices were estimated using the Dice coefficient of similarity [32]. The value of 1.00 indicates that the two samples are identical and the value of 0 indicates that the samples are dissimilar. Cluster analyses were carried out on similarity estimates using unweighted pair-group method to produce a dendrogram using arithmetic average (UPGMA) in the NTSYSpc-verson 1.80 software program [33].

3. RESULTS AND DISCUSSION

Twenty populations collected from different locations in South India including 2 commercial North Indian cultivars (WS-02 and -07) were maintained under uniform growth conditions. The collections of different habitats for RAPD analysis are listed in Table 1 and their morphological characteristics like color, shape, tip and size of leaf, nature of stem, height of plant, node space, color of fruits and size and nature of root are listed in Table 2. There were polymorphic differences among these 20 populations, but their variations were not distinctive except some populations. Therefore, it is difficult to differentiate them in groups based on the morphological characteristics only.

Therefore, the RAPD analysis was applied to find the molecular differences among morphologically indistinguishable variants of *W. somnifera* populations. Primers (OPA01, OPA02, OPA07, OPA08, OPA10, OPA17, OPB01, OPB05, OPB07, OPB08 and OPB12) were selected on the basis of profiles with each of the template DNA tested. The 11 primers showed the good amplification of polymorphic bands (Table 3), but another 12 random primers did not give any amplification products. The other 17 primers showed the amplification of products but the intensity of the fragments was very low. Some primers produce highly polymorphic patterns among the populations, while others generate little polymorphic or monomorphic patterns. The amplification profiles of total genomic DNA from 20 populations with 11 random primers produced 96 consistent RAPD markers, ranging in size from 0.2 kb to 4.0 kb; out of which 21 were monomorphic (21.9%) and 75 were polymorphic (78.1%).

The number of DNA fragments produced ranged from 5 to 12 with the primers OPA02 and OPB01, respectively. Pattern of RAPD profiles produced by the primers OPA08 and OPB01 were shown in Fig. 2. Among the fragments amplified by primer OPA01, 1 unique band of 0.4 kb was present in population WS-12 which clearly distinguished it from other populations of *W. somnifera.* Similarly, another unique band of 1.5 kb was observed in WS-05 which

produced 5 amplified bands with the primer OPA02. Two unique bands (0.9 kb and 1.8 kb) and 1 unique band (0.4 kb) were observed respectively in WS-03 and -14 with the primer OPA07. This observation clearly indicates that these 2 populations were different from other varieties. Two unique bands (0.4 kb and 0.9 kb) were observed in WS-20 with the primer OPA08. The results also indicate that the primer OPA10 produced 2 unique bands (1.7 kb and 1.5 kb) in WS-01 and -12, which were different from other collections. The primer OPA17 produced 8 amplified bands, which were polymorphic (100%). The primer OPB01 produced 12 amplified bands out of which 8 bands were polymorphic (66.7%) and 4 bands were monomorphic (33.3%) including 2 unique bands (0.35 kb and 2.3 kb) appeared in WS-03 and -09, respectively. The primer OPB05 produced 6 amplified bands including 1 unique band having 1.2 kb in WS-14. Two unique bands (1.2 kb and 2.0 kb) appeared in WS-03 and -14 with the primer OPB07. The primer OPB08 produced 8 amplified bands out of which 5 bands were polymorphic (62.5%) and 3 bands were monomorphic (37.5%). The primer OPB12 produced 10 amplified bands including 2 unique bands of 0.7 kb and 0.9 kb in WS-14 and -15. More numbers of unique band were observed in WS-14 with 11 primers. This clearly indicated that WS-14 was different from other populations. The primers OPA08, OPA17, OPB07 and OPB12 showed the most distinguished fragments in different populations of *W. somnifera.*

Thus, RAPD analysis indicates the occurrence of pronounced genetic variations among different collections of *W. somnifera* in South India. This may be due to decreased gene flow because of increased geographic isolation caused by human destruction of native/natural habitat. Accordingly, pronounced genetic differentiation among populations has been reported for a number of rare species [34,35]. The observed genetic differentiation among the populations of *W. somnifera* in South India suggests the low gene flow in accordance with the geographic isolation of the populations.

Similarity coefficients obtained by RAPD profile is shown in Table 4. AFLP analysis of genetic variation and relationship among and within *Withania* species from North India was reported [18]. Similar type of results were also reported in other plants like *Artemisia annua* using inter simple sequence repeats (ISSR) and random amplification of polymorphic deoxyribonucleic acid (RAPD) markers [36] and *Trigonella foenum* using amplified fragment length polymorphism (AFLP) markers [37]. The results were observed in the present study that showed similarity coefficient ranges from 0.53 to 0.98 and the variations among 20 different populations of *W. somnifera*. These similarity coefficients were used to generate a dendrogram tree for cluster analysis using UPGMA method, which provides an idea about the genetic relationship between the populations. The cluster analysis indicates the presence of 5 major groups among the 20 populations of *W. somnifera* tested (Fig. 3). The major groups (a) and (c) had only one population i.e. WS-14 and -20, respectively. The population of WS-14 possesses only 64% of genetic similarity with other populations. The cluster analysis clearly indicates that WS-14 is grouped in a separate cluster (a), suggesting that the WS-14 population has the higher level of genetic variation compared with other South Indian populations and North varieties WS-02 and -07. The major groups (b) and (d) have two members in the population i.e. WS-02 and -03 and WS-09 and -12, respectively. Another major group (e) is represented by 14 populations such as WS-01, -04, 05, -06, -07, -08, -10, -11, -13, -15, -16, -17, -18 and -19. Among the 20 populations, WS-04 and -17 showed the highest similarity indices (98%), while WS-03 and -18 showed the lowest similarity indices (54%).

Table 2. Morphological variations in leaf and other characteristics of twenty different populations of *W. somnifera.*

Accession number	Leaf characteristics			Other characteristics					
	Colour	Shape	Tip	Size (cm^2)	Nature of stem	Height of plant	Node space	Colour of fruit	Size and nature of root
WS-01	Dark green	Ovate	Acute	25-40	Hard	3-4 ft	Small	Red	Big, Hard
WS-02	Dark green	Ovate	Acute	>40	Hard	4-5 ft	Long	Red	Big, Hard
WS-03	Dark green	Ovate	Acute	>40	Hard	3-4 ft	Long	Red	Big, Hard
WS-04	Dark green	Ovate	Acute	>40	Hard	3-4 ft	Long	Red	Big, Semi hard
WS-05	Dark green	Ovate	Acute	25-40	Hard	3-4 ft	Small	Red	Big, Semi hard
WS-06	Dark green	Ovate	Acute	>40	Hard	4-5 ft	Long	Red	Big, Hard
WS-07	Dark green	Ovate	Acute	>40	Hard	3-4 ft	Small	Red	Big, Hard
WS-08	Light green	Elliptic	Acute	<25	Semi hard	2-3 ft	Small	Orange	Small, Semi hard
WS-09	Dark green	Ovate	Acute	>40	Hard	4-5 ft	Long	Red	Big, Hard
WS-10	Dark green	Ovate	Acute	25-40	Hard	3-4 ft	Small	Red	Big, Hard
WS-11	Dark green	Ovate	Acute	>40	Semi hard	4-5 ft	Long	Red	Big, Semi hard
WS-12	Dark green	Elliptic	Acute	25-40	Semi hard	2-3 ft	Small	Red	Big, Semi hard
WS-13	Dark green	Ovate	Acute	25-40	Hard	3-4 ft	Small	Red	Big, Hard
WS-14	Light green	Elliptic	Blunt	<25	Semi hard	2-3 ft	Small	Orange	Small, Semi hard
WS-15	Light green	Elliptic	Acute	25-40	Hard	3-4 ft	Small	Red	Big, Hard
WS-16	Dark green	Ovate	Blunt	25-40	Hard	2-3 ft	Small	Red	Big, Hard
WS-17	Dark green	Ovate	Acute	25-40	Hard	4-5 ft	Small	Red	Big, Hard
WS-18	Light green	Elliptic	Blunt	<25	Semi hard	2-3 ft	Small	Red	Small, Semi hard
WS-19	Light green	Elliptic	Acute	25-40	Hard	3-4 ft	Small	Red	Big, Hard
WS-20	Dark green	Ovate	Acute	>40	Hard	4-5 ft	Long	Red	Big, Hard

Some South Indian populations such as WS-03 and -06 have genetic similarities with North varieties WS-02 and -07, respectively. WS-03 is a South Indian population collected from the plains of Perambalur at Tamilnadu, but WS-02 is a North Indian variety collected from CIMAP-Lucknow. Nevertheless, WS-02 and -03 were grouped into a single cluster (b). Similarly, WS-08 and -10 were grouped into a single cluster (e) and showed 85% similarity indices, but both populations were collected from different regions far away from each other (Maharastra and Puducherry). In addition, the populations collected from different states of South India like Tamilnadu (WS-04, -05, -11, and -17), Andhra Pradesh (WS-13), Kerala (WS-18), Karnataka (WS-19) were grouped in the same cluster (e). It is difficult to figure out how these populations from different states grouped within the same cluster. On the other hand, WS-01 and WS-03 were not grouped into a single cluster, but both populations were collected from the plain places (natural uncultivated lands) of Tamilnadu.

Table 3. Total number of amplified fragments and number of polymorphic bands generated by PCR using selected random decamer in 20 different populations of *W. somnifera*

Primer	Sequence	Total amplified products	Polymorphic products	Polymorphism (%)	Size range (Kb)
OPA01	CAGGCCCTTC	9	6	66.7	0.4 – 2.0
OPA02	TGCCGAGCTG	5	2	40.0	0.8 – 1.6
OPA07	GAAACGGGTG	10	6	60.0	0.2 – 1.8
OPA08	GTGACGTAGG	11	11	100.0	0.4 – 4.0
OPA10	GTGATCGCAG	11	10	90.9	0.6 – 2.3
OPA17	GACCGCTTGT	8	8	100.0	0.6 – 1.6
OPB01	GTTTCGCTCC	12	8	66.7	0.3 – 2.3
OPB05	TGCGCCCTTC	6	3	50.0	0.6 – 2.1
OPB07	GGTGACGCAG	6	6	100.0	0.7 – 2.0
OPB08	GTCCACACGG	8	5	62.5	0.6 – 2.4
OPB12	CCTTGACGCA	10	10	100.0	0.7 – 2.3

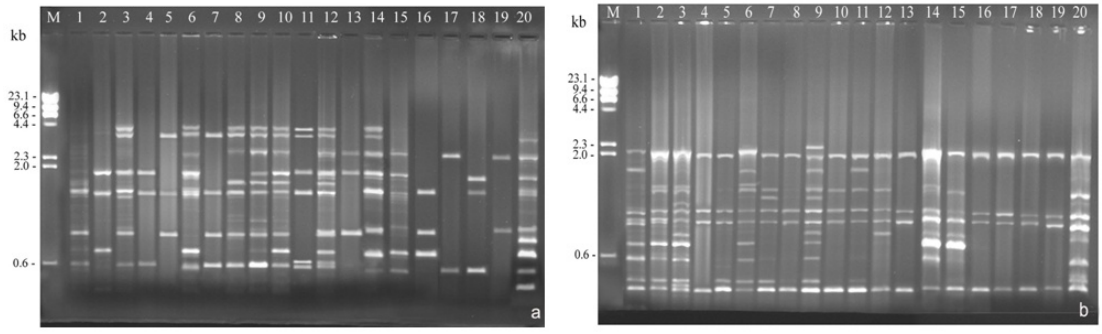

Fig. 2. RAPD patterns of 20 different populations of *W. somnifera* (L.) Dunal
a) the primer OPA8 (5' GTGACGTAGG 3') and b) the primer OPB01 (5' GTTTCGCTCC 3')

Table 4. Similarity matrix for Nei and Li's coefficient of a total of twenty different populations of *W. somnifera.*

	WS-01	WS-02	WS-03	WS-04	WS-05	WS-06	WS-07	WS-08	WS-09	WS-10	WS-11	WS-12	WS-13	WS-14	WS-15	WS-16	WS-17	WS-18	WS-19	WS-20
WS-01	1.00																			
WS-02	0.72	1.00																		
WS-03	0.66	0.75	1.00																	
WS-04	0.71	0.65	0.61	1.00																
WS-05	0.69	0.79	0.57	0.75	1.00															
WS-06	0.62	0.69	0.57	0.65	0.69	1.00														
WS-07	0.62	0.78	0.69	0.71	0.76	0.83	1.00													
WS-08	0.66	0.69	0.63	0.73	0.84	0.73	0.69	1.00												
WS-09	0.62	0.68	0.56	0.56	0.67	0.72	0.69	0.83	1.00											
WS-10	0.65	0.67	0.61	0.80	0.75	0.73	0.74	0.85	0.78	1.00										
WS-11	0.86	0.76	0.58	0.95	0.92	0.71	0.82	0.82	0.68	0.76	1.00									
WS-12	0.61	0.68	0.64	0.75	0.69	0.75	0.72	0.77	0.77	0.79	0.63	1.00								
WS-13	0.69	0.81	0.61	0.84	0.94	0.75	0.86	0.78	0.64	0.69	0.93	0.58	1.00							
WS-14	0.58	0.64	0.64	0.58	0.65	0.61	0.57	0.65	0.60	0.58	0.55	0.60	0.75	1.00						
WS-15	0.62	0.64	0.53	0.75	0.73	0.65	0.69	0.71	0.70	0.69	0.76	0.66	0.82	0.67	1.00					
WS-16	0.94	0.76	0.71	0.88	0.88	0.94	0.88	0.88	0.82	0.88	0.88	0.88	0.82	0.82	0.82	1.00				
WS-17	0.82	0.76	0.58	0.98	0.88	0.94	0.82	0.88	0.82	0.84	0.95	0.88	0.94	0.71	0.94	0.88	1.00			
WS-18	0.85	0.73	0.54	0.95	0.88	0.94	0.80	0.85	0.80	0.88	0.96	0.77	0.94	0.81	0.92	0.88	0.95	1.00		
WS-19	0.72	0.75	0.57	0.97	0.88	0.94	0.82	0.82	0.75	0.93	0.95	0.68	0.94	0.61	0.94	0.88	0.98	0.95	1.00	
WS-20	0.65	0.67	0.61	0.59	0.63	0.74	0.65	0.67	0.75	0.64	0.63	0.68	0.61	0.59	0.63	0.82	0.82	0.77	0.75	1.00

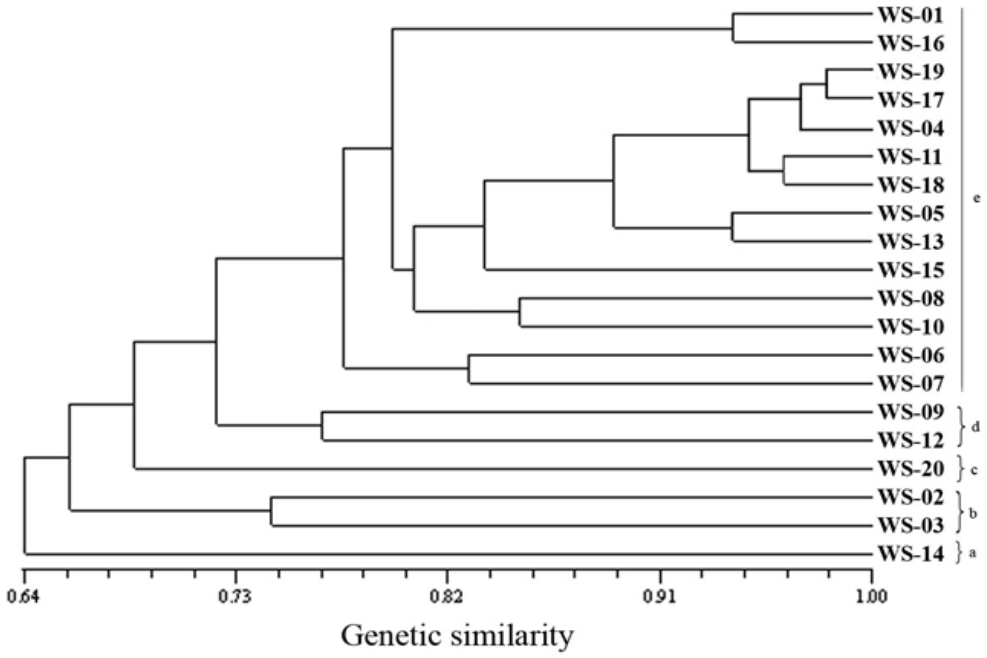

Fig. 3. UPGMA dendrogram showing the genetic relationships within 20 different populations of *W. somnifera* (L.) Dunal

4. CONCLUSION

It is interesting to note that the populations of *W. somnifera* collected from same region shows genetic variation, but the populations from different region show genetic similarities. The dendrogram tree in the present study does not support geographic clustering of the populations of *W. somnifera*, thereby indicating that the clustering is independent of state (geographic) origin. It is logical to hypothesize that the genetic variation of *W. somnifera* populations in this study may have been due to their natural hybridization within populations and selection pressure during the course of evolution. It is assumed that *W. somnifera* may evolve from one of the locations, then migrate to long distance, and then adapt in that location by interacting with environmental factors prevailing over there. This may be the causes for genetic diversity which may influence and produce changes in phytochemical constituents of *W. somnifera* populations. It evident that the RAPD markers showed the promise in elucidating patterns of genetic variation among the populations of *W. somnifera* in South India, which may useful in the genetic improvement and conservation of *W. somnifera*.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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