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Original Article

Genetic variability and relationship studies of Ber *Ziziphus nummularia* (Burm. F.) using morphological and molecular markers

Yashmin Akhtar, Ravish Choudhary, Kailash Chandra Sharma and Manju Sharma*

ABSTRACT

Introduction: Ber (*Ziziphus nummularia* (Burm. F.) is the most commonly occurring branched thorny shrub species in the Indian desert. A total of 10 Zadber accessions collected from different agroclimatic regions of India, were evaluated for phenotypic and genotypic variations using morphological and inter-simple sequence repeats (ISSR) markers. **Materials And Methods:** Morphological characterization was done using descriptors and Molecular characterization studies was done with fifty primers of University of British Columbia (UBC) procured from Geno Biosciences Pvt. Ltd., were used for ISSR-PCR optimisation trials. Eleven primers, which gave the best amplification results with the sample DNA, were selected for final ISSR-PCR analysis. **Results:** Significant variability was observed in the selected Zadber accessions by the analysis of five quantitative and 25 qualitative morphological characters of leaves, fruits and seeds. ISSR markers also showed polymorphism (86.58%). Jaccard's genetic similarity value of ISSR was found in the range of 0.45-0.77 (average 0.61) suggesting moderate level of genetic diversity within the Zadber group. Two of the eleven ISSR primers were also able to generate cultivar specific amplicons, which may be used for identification of accessions Zadber-5 and Zadber-8. **Conclusion:** The present study revealed that morphological and molecular markers can be successfully utilized for determining genetic diversity and genetic relationship of Zadber cultivars and used in breeding programmes.

Key words: Zadber, Morphology, ISSR, Genetic variability, UPGMA.

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INTRODUCTION

Zadber (*Ziziphus nummularia* (Burm.f.) belongs to the Rhamnaceae family. Of the well-known species of the genus Ziziphus, ber (*Z. nummularia* (Burm.f.) is the most common in the tropical and sub-tropical regions. These species are indigenous to North Africa, Afghanistan, North India, Southern China, Malaysia and Queensland in Australia. Zadber is now widely distributed and has become naturalized in tropical Africa, Iran, Syria, Sri Lanka and part of the Mediterranean.[1] Zadber can provide food security, due to sustained production of the fruit, irrespective of drought, as the tree is drought and saline tolerant and can grow on poor degraded land.[2] Zadber fruits are very nutritious and usually eaten fresh. The fruits are a drupe, varying from round to elongate and from cherry-size to plum-size depending on cultivar.[3] The development study by markers and their polymorphic information represent a significant improvement in Chinese jujube genomic resources and will facilitate both genetic and breeding applications, for the development of new cultivars.[4] The Zadber cultivars were varying in fruit physio-chemical characteristics. Genetic manipulation in Zadber varieties done by making divergent crosses to develop potential varieties of Zadber for lac production.[5] Fruit weight ranged from 3.8 to 39.5 mg; fruit length ranged from 1.82 to 5.80 cm, diameter 1.1 to 4.7 cm. Fresh mature Zadber fruits contains 81 to 97% pulp.[6,7] Zadber pulp contains 12-23% TSS, 0.13-1.42% acidity, 3.1-14.5% total sugars, 1.4-9.7% reducing sugars, 5.6% sucrose, 1.5% glucose, 2.1% fructose and 1.0% starch.[8] Zadber pulp is a rich source of vitamin C.[9] reported that ascorbic acid content in different cultivars ranged from 39-166 mg/100 g of pulp. Significant differences in allele frequency distributions and in genetic diversity parameters between the core accessions and the 622 genetically unique accessions done by[10]. Molecular markers techniques have been developed to analyze and estimate genetic diversity in plant species. Among the various marker systems, the randomly amplified polymorphic DNA (RAPD) is one of the most popular DNA-based approaches.[11-14] The ability of Ziziphus species and different varieties of mauritiana has study to cross freely to build up of rich gene pool that depicts heterozygosity in their adaptability to soil and climate; morphological, physiological and phenological traits; chromosome number; tolerance/resistance to biotic and abiotic stresses and genomic DNA.[15] The RAPD technique is a potentially simple, rapid, reliable and effective. ISSR is more reproducible than the RAPD technique and is preferred. The ISSR markers are generated from single-primer PCR reactions and the primer is designed from

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di-or trinucleotide repeat motifs with a 5 or 3 anchoring sequence of one to three nucleotides.[16] The analysis using the ISSR markers may be used for evaluation of the genetic diversity due to their efficiency in revealing polymorphism closely related to germplasm that help in Ziziphus genome analysis.[17] ISSR technique producing a high degree of polymorphism, generating reliable information for DNA analysis with the necessary sensibility to distinguish among individuals genetically related. ISSR analysis is technically simpler than many other marker systems. The method provides highly reproducible results and generates abundant polymorphisms in many systems. The leaves are antipyretic and reduce obesity. The fruit is cooling, tonic, digestible, laxative aphrodisiac and removes biliousness, thirst, vomiting and burning sensations.[18] The dried fruits contain alkaloids, triterpenoids and saponins. They are anticancer, anodyne, refrigerant, sedative, pectorial, styptic, stomachic and tonic. They are used to purify the blood and aid digestion.[19] The cross- pollinating nature and seed propagation are the major reasons of very high intra specific genetic variability in ber may be harnessed for selection and genetic improvement (Singh *et al* 2006). The PCR technology has led to the development of two simple and quick techniques viz., random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats ISSR.[20] The present study based on difference in the morphological and molecular characters of 10 accessions of *Ziziphus nummularia* belonging to different states (Rajasthan, Delhi and Gujarat). Molecular markers based on polymerase chain reaction (PCR) method used to study morphological and genetic characteristics. We report here the genetic diversity assessed using RAPD and ISSR markers with the comparison of the two marker systems to evaluate the genetic relationship among the 10 accessions belonging to *Z. nummularia* on the basis of leaf sample.

MATERIALS AND METHODS

A total of 10 accessions of Zadber (*Ziziphus numuularia*) were collected from different agroclimatic zones of India which belong to three different states (Delhi, Rajasthan and Gujarat) and used for morphological and molecular studies (Table 1 and Figure 1). Selective sampling strategy was employed, where samples collected from single plant and treated as individual accession. Leaf and fruit samples of each accession were collected for confirmation of taxonomic identity, characterization and DNA extraction.

Morphological Characterization

Morphological characterization of 10 accessions of *Ziziphus nummularia* was done using descriptors. Characterization data of 30 characters (25 qualitative and 05 quantitative) of leaf, fruit and seed were recorded for the collected germplasm (Table 2). A pair-wise similarity matrix was generated based on simple matrix coefficient method using software NTSYS-pc ver. 2.1.[22] A cluster analysis was performed using the Unweighed Pair Group Method with Arithmetic average (UPGMA) based on simple matching coefficient in NTSYS software. Principal Component Analysis (PCA) was also carried out to study correlations among the variables and establish relationships among cultivars using the same software. Two-way Mantel test[23] for goodness of fit for UPGMA cluster was also performed using the same software.

Table 1: List of Zadber collected from different states of India

Accession no	District	State	Latitude	Longitude
Zadber-1	Jodhpur	Rajasthan	26.25	73.02
Zadber-2	Nagaur	Rajasthan	27.20	73.74
Zadber-3	Pilani	Rajasthan	28.37	75.60
Zadber-4	New Delhi	Delhi	28.65	77.23
Zadber-5	Jaipur	Rajasthan	26.93	75.79
Zadber-6	Rajgarh	Rajasthan	28.30	74.95
Zadber-7	Vadodra	Gujarat	22.31	73.17
Zadber-8	Tonk	Rajasthan	26.17	75.78
Zadber-9	Jhalawar	Rajasthan	24.60	76.16
Zadber-10	Bharatpur	Rajasthan	27.22	77.49

Figure 1: (a) Habit of Plant, diversity in (b) Leaf samples and (c) Fruit samples

Genomic DNA extraction and purification

Total genomic DNA was extracted from all the 10 accesions of *Ziziphus nummularia* through Cetyl Tri-methyl Ammonium Bromide (CTAB) method.[24] Quantitation of isolated DNA was done spectrophotometrically and its quality checked by electrophoresis on 0.8% Agarose gel.

ISSR-PCR amplification

A total of fifty primers of University of British Columbia (UBC)[25] procured from Geno Biosciences Pvt. Ltd., were used for ISSR-PCR optimisation trials. Eleven primers, which gave the best amplification results with the sample DNA, were selected for final ISSR-PCR analysis. PCR-amplification was carried out in 25 µl reaction volume containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.0-2.5 mM $MgCl_2$, 0.2 mM dNTP each, 1.0 U Taq DNA polymerase (Bangalore Genie, India), 0.2 µM primer and 25-30 ng genomic DNA. The amplification was performed in a PTC–200 Thermocycler (MJ Research, Massachusetts, USA), with reaction conditions programmed as initial pre-denaturation at 94°C for 4 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 50°Cfor 1 min, and extension at 72°C for 2 min. A final 7 min extension at 72°C followed the completion of 35 cycles. Amplification products were separated by electrophoresis on 1.5% Agarose gel stained with ethidium bromide at 100 V for 3 hours, and bands were visualized and documented in AlphaImager® HP (Alpha Innotech Corporation).

Data analysis

Amplified bands generated from ISSR PCR amplification were scored based on the presence (1) or absence (0) of bands for each primer and used to calculate a genetic similarity matrix employing the simple

Table 2: Qualitative and quantitative characters selected for the present study

S.No.	Characters	Zadber-1	Zadber-2	Zadber-3	Zadber-4	Zadber-5	Zadber-6	Zadber-7	Zadber-8	Zadber-9	Zadber-10
1	Status of Sample	wild	wild	wild	Wild	wild	wild	wild	wild	wild	wild
2	Growth Habit	spreading	spreading	upright	upright	upright	spreading	upright	spreading	spreading	spreading
3	Growth and vigour	medium	medium	strong	strong	strong	medium	strong	weak	medium	medium
4	Plant type	shurb	shurb	shurb	Tree	shurb	shurb	shurb	shurb	shurb	Shrub
5	Leaf Length(cm.)	2.9	0.7	2.3	4.4	3.3	1.3	2.9	1.7	1.8	1.3
6	Leaf Width (cm)	1.5	0.5	1.5	1.9	2.2	1	1.5	1.5	1.3	0.9
7	Leaf Colour	green	green	green	green	green	green	green	green	green	Green
8	Leaf Shape	ovate	ovate	ovate	ovate	ovate	ovate	ovate	ovate	ovate	Ovate
9	Leaf Edge	entire	entire	entire	entire	entire	entire	entire	entire	entire	Entire
10	Leaf Apex	obtuse	obtuse	obtuse	obtuse	obtuse	obtuse	obtuse	obtuse	obtuse	Obtuse
11	Phyllotaxy	alternate	alternate	alternate	alternate	alternate	alternate	alternate	alternate	alternate	Alternate
12	Branching	zigzag	zigzag	zigzag	zigzag	zigzag	zigzag	zigzag	zigzag	zigzag	Zigzag
	Fruit	Globose	Globose	Globose	Globose	Globose	Globose	Globose	Globose	Globose	Globose
13	Fruit Length(mm)	17.1	9.5	17.4	14.6	14.3	10.2	17.1	9.4	9.8	10
14	Fruit diameter (mm)	16.3	9.7	13.6	11	10.6	9.5	16.3	9	7.8	9.9
15	Fruit weight(gm)	1.26	0.35	1.02	0.67	0.66	0.31	1.26	0.23	0.19	0.24
16	Fruit Colour	Red	Red	Red	Red2	Red	Red	Red	Red	Red	Red
17	Fruit Shape	Spheroid	Spheroid	ovate	Spheroid	ovate	Spheroid	Spheroid	Spheroid	Spheroid	Spheroid
18	Fruit Size	large	small	medium	medium	medium	small	medium	small	small	Small
19	Fruit Juiciness	very juicy	juicy	juicy	Juicy	juicy	juicy	juicy	juicy	juicy	Juicy
20	Fruit Setting	high	medium	medium	medium	high	high	light	medium	light	Medium
21	Fruiting Season	late	late	late	Late	late	late	late	late	late	Late
22	Fruit Taste	sweet	sweet	sweet	sweet	sweet	sweet	sweet	sweet	sweet	Sweet
23	Skin Colour	red	red	red	Red	red	red	red	red	red	Red
24	Skin Thickness	thin	thin	thin	Thin	thin	thin	thin	thin	thin	Th i n
25	Flesh Thickness	thick	mdium	medium	medium	medium	medium	medium	medium	medium	Medium
26	Flesh Aroma	strong	strong	strong	strong	strong	strong	strong	strong	strong	Strong
27	Flesh Texture	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth
28	Flowering	spare	spare	spare	spare	spare	spare	spare	spare	spare	Spare
29	Bearing	heavy	heavy	heavy	heavy	heavy	heavy	heavy	heavy	heavy	Heavy
30	Flower colour	white	white	white	white	white	white	white	white	white	White

matching coefficient using software NTSYS-pc version 2.1.[22] Cluster analysis was performed on both morphological and molecular data using the "Unweighted Pair Group Method Using Arithmetic Means" (UPGMA) algorithm, from which dendrograms depicting similarity among varieties were drawn and plotted using NTSYS-pc software. The cophenetic correlation was calculated in order to find the degree of association between the original similarity matrix and the tree matrix in both morphological and molecular analysis. Comparison between both the methods was performed for the accessions for which both data were available by calculating the correlation between the two data sets using the Mantel test in NTSYS-pc. Principal component analysis (PCA) was also carried out to find out genetic association among the cultivars using same software.

RESULTS

Morphological analysis

All the accessions of Zadber collected from three different states of India, were generally morphologically similar, however, some of the characters showed variation. Comparative analysis of thirty-three morphological characters studied revealed moderate variation among them. A pair-wise similarity among the cultivars of Zadber ranged from 0.27 to 0.62 with an average of 0.44 based on morpho-metric data. Maximum similarity was observed between Zadber-2 and Zadber-4 accessions, while minimum was observed Zadber-7 accessions. A dendrogram was generated based on morpho-metric data in which two major clusters were formed for the ten accessions under study (Figureure 2). The first cluster comprised of four accessions, namely, Zadber -1, Zadber -3, Zadber -8 and Zadber -10. Within this cluster Zadber -1 and Zadber -3 were most similar to each other morphologically with similarity value of 0.54. The second cluster was the biggest one comprising of 6 accessions, viz. Zadber -2, Zadber -4, Zadber -5, Zadber -6, Zadber -7 and Zadber -9. Within this cluster, the accessions Zadber -2 and Zadber -4 were highly similar morphologically showing a similarity of 0.62 while Zadber -7 was morphologically very distinct from remaining nine accessions having a similarity value of 0.27.

Two- dimensional plot (2-D plot) generated from PCA showed three groups which was found almost similar to the clustering pattern of UPGMA dendrogram. In 2-D plot, the accession Zadber-10 was grouped together with Zadber -2, Zadber-4, Zadber-5, Zadber-6 and Zadber-9 in 2-D plot but in the dendrogram it was grouped along with Zadber-1, Zadber-3 and Zadber-8. Whereas Zadber-7 formed separate group in 2-D plot but in the dendrogram it was grouped along with Zadber-2, Zadber-4, Zadber-5, Zadber-6 and Zadber-9 (Figure 3). The analysis gave first 8 principal components, which contributed 99.94% of the total variability of the collected accessions. The first 5 principal components accounted for 96.74% of the total variability and the first three accounted for 85.46% of the variance, in which maximum variability was contributed by first component (48.69%) followed by 2nd component (25.70%), and 3rd component (11.06%) (Table 3).

ISSR analysis

Eleven primers were selected for the ISSR analysis based on the reproducibility and banding patterns. A total of 98 bands were generated, of which 86 bands were polymorphic (86.58%). Each primer amplified 4-12 polymorphic amplicons with an average of 7.8 amplicons per primer. UBC-894 primer amplified the maximum number of 12 amplicons, whereas UBC-829 amplified the lowest number of polymorphic amplicons 4. Figure 4 showed representative ISSR profile generated by primer UBC-809 and UBC-894. The polymorphism percentage ranged from 57.14% (primer UBC-829) to 100% (UBC-814, UBC-840, UBC-841, UBC-855 and UBC-894). Average polymorphism across all the 10 accessions was 86.58%. Overall size of the PCR amplified fragments ranged from 250bp to 1200bp (Table 2). Pattern of distribution of bands across all cultivars of *Z. nummularia* revealed that the primer UBC-809 (660bp, 730bp) for Zadber-5 and UBC-894 (910 bp) for Zadber-8 amplified a unique DNA fragment which distinguished one cultivar from the others (Table 4).

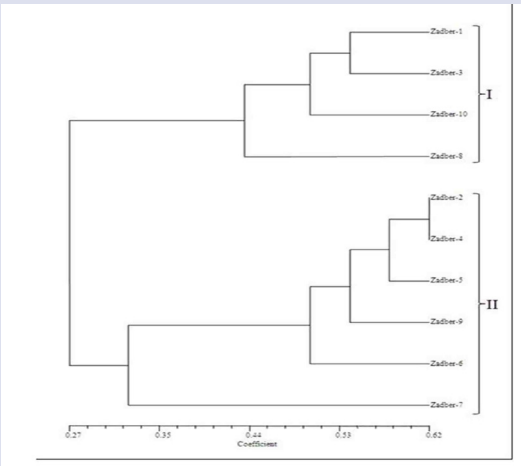

Figure 2: UPGMA dendrogram of 10 Zadber accessions generated based on morphomatric data

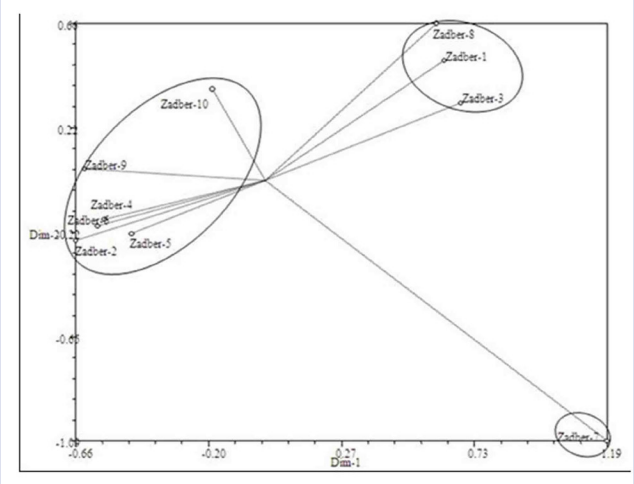

Figure 3: 2-D plot of 10 Zadber accessions generated based on morphomatric data

Table 3: Eigenvectors of morphological variables explained by first three principal

S. No	Eigenvalue	Percent	Cumulative
1	4.382308	48.6923	48.6923
2	2.313219	25.7024	74.3947
3	0.995603	11.0623	85.457
4	0.646846	7.1872	92.6442
5	0.367987	4.0887	96.7329
6	0.22346	2.4829	99.2158
7	0.056177	0.6242	99.84
8	0.008532	0.0948	99.9348

Table: 4 Details of amplified bands generated by 11 ISSR primers in 10 accessions of Zadber

Si. No	Primer code	Sequence 5'-3'	Total no. of bands	PBa	PPB"	Amplified fragment length
1.	UBC-809	AGAGAGAGAGAGAGAGG	08	06	75.00	625-960
2.	UBC-814	CTCTCTCTCTCTCTCTA	09	09	100	325-960
3.	UBC-825	ACACACACACACACACT	08	06	75.00	550-1200
4.	UBC-829	TGTGTGTGTGTGTGTGC	07	04	57.14	325-940
5.	UBC-840	GAGAGAGAGAGAGAGACTT	09	09	100	325-970
6.	UBC-841	GAGAGAGAGAGAGAGACTC	09	09	100	300-800
7.	UBC- 850	GTGTGTGTGTGTGTGTCTC	09	07	77.78	500-980
8.	UBC- 854	TCTCTCTCTCTCTCTCAGG	08	07	87.50	450-1200
9.	UBC-855	ACACACACACACACACCTT	09	09	100	380-910
10.	UBC-894	TGGTAGCTCTTGTCAGGCAC	12	12	100	300-1270
11.	UBC-895	AGAGTTGGTAGCTCITGATC	10	08	80.00	250-1170
		Total	98	86	86.58 %	

a Total Polymorphic Bands, b Percentage of Polymorphic Bands

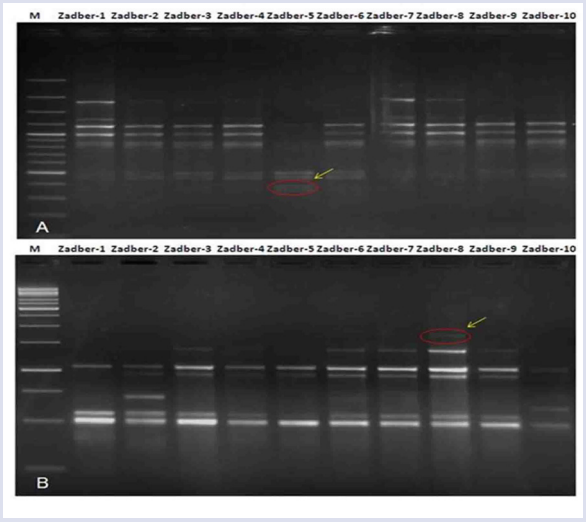

Figure 4: Gel profiles of the 10 *Ziziphus nummularia* accessions generated with the ISSR primers: [A] UBC-809 and [B] UBC-894. M is the λ DNA marker. Arrows shows the unique band in red circle

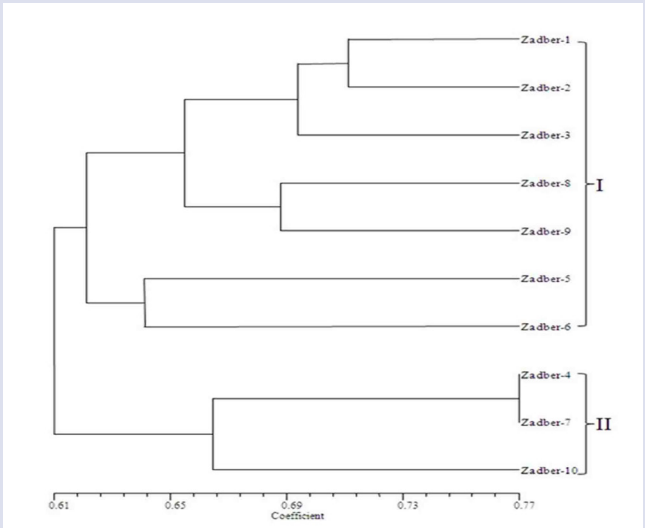

Figure 5: UPGMA dendrogram of 10 *Ziziphus nummularia* accessions generated based on ISSR data

Genetic diversity and relationships

A pairwise similarity co-efficient among all the 10 accessions of *Z. nummularia* ranged from 0.45-0.77. The maximum similarity of 0.77 was observed between accessions Zadber-4 and Zadber-7, indicating that they are genetically most similar, whereas Zadber -2 and Zadber -10 showed least similarity coefficient of 0.45. Average similarity across all the cultivars was 0.61. In the dendrogram all the 10 accessions were grouped into two major clusters (Figure 5). First cluster was the biggest one comprising of seven accessions viz. Zadber -1, Zadber -2, Zadber -3, Zadber-5, Zadber-6, Zadber-8 and Zadber-9. Within this cluster Zadber-1 and Zadber-2 are genetically most similar with value of 0.71 while Zadber -5 and Zadber-6 were most distinct from remaining accessions with similarity value of 0.64. The second cluster comprised of three accessions viz. Zadber -4, Zadber -7 and Zadber-10 in which Zadber-4 and Zadber-7 was most similar with 77% similarity from all other accessions and Zadber-10 was distinct with similarity value of 0.66.

Based on Mantel Z-statistics,[26] the correlation coefficient (r) was estimated to be 0.64. 2-D plot generated from PCA of ISSR data also in coherence with the clustering pattern of UPGMA dendrogram, except Zadber-9 was grouped together with Zadber-1, Zadber-2, Zadber-3, Zadber-5, Zadber-6 and Zadber-8 in UPGMA dendrogram but in 2D plot it was grouped along with Zadber-4, Zadber-7 and Zadber-10. Whereas Zadber -5 formed separate group in 2-D plot but in the dendrogram it was grouped along with Zadber-1, Zadber-2, Zadber-3, Zadber-6, Zadber-8 and Zadber-9 (Figure 6). The analysis gave first 8 principal components, which contributed 94.33% of the total variability of the collected accessions. The first 5 principal components accounted for 69.93% of the total variability and the first three accounted for 47.34% of the variance, in which maximum variability was contributed by first component (18.95%) followed by 2nd component (15.82%), and 3rd component (12.56%) (Table 5).

Table 5: Eigenvectors explained by first three principal components based on ISSR data

Sl.No.	Eigenvalue	Percent	Cumulative
1	1.705854	18.9539	18.9539
2	1.424093	15.8233	34.7772
3	1.130297	12.5589	47.336
4	1.05444	11.716	59.052
5	0.979193	10.8799	69.932
6	0.892369	9.9152	79.8472
7	0.713922	7.9325	87.7796
8	0.589524	6.5503	94.3299

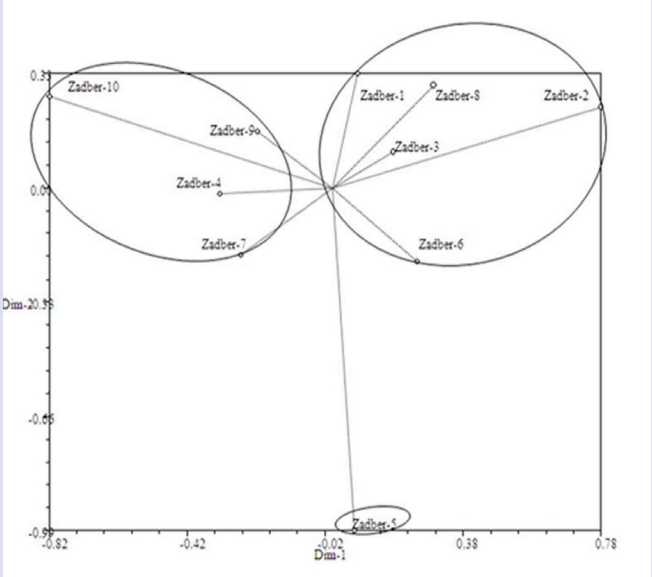

Figure 6: 2-D plot of 10 *Ziziphus nummularia* accessions generated based on ISSR data

DISCUSSION

Experiments with Zadber accessions have demonstrated the potential of ISSR markers as a rapid, reproducible and useful method and clustering into different groups. The high level of genetic polymorphism (86.58%) was observed among the 10 accessions of Zadber based on 11 primers. This could be explained by the fact that somatic mutations are the main source of variability in accessions of this species. However, high level of polymorphism (86.20%) was reported between Zadber based on RAPD markers.[21] Pair wise similarity analysis of 33 morphological characters in the 10 accessions of Zadber revealed that maximum similarity (0.62) occurred between Zadber-2 and Zadber-4 accessions. Minimum similarity (0.27) was observed in Zadber-7 accessions this may be attributed to their different origin (Gujarat) where they have developed their distinct characters. The average similarity value of 0.44 indicated that accessions show moderate to significant variability among these accessions with respect to morphological traits. The maximum similarity of 0.77 was observed between accessions Zadber-4 and Zadber-7, indicating that they are genetically most similar, whereas Zadber-2 and Zadber-10 showed least similarity coefficient of 0.45. Average similarity across all the cultivars was 0.61. In the dendrogram, all the 10 accessions were grouped into two major clusters. Based on ISSR markers, high similarity value of 0.77 was found between two accessions, Zadber-4 and Zadber-7 showing very close genetic relationship between them and this may be due to their common origin by mutation. Low genetic similarity (0.45) between cultivars Zadber-2 and Zadber-10 may be due to different sources of origin of these accessions. High genetic similarity (avg. 0.61) was recorded within this group, which showed narrow level of genetic diversity existed within Zadber. This was also congruent with high level of polymorphism occurred within this group. Similar reports were found with high genetic variation among the cultivars of Zadber based on RAPD markers.[21] A search for unique bands was made for all the accessions tested, in which, UBC-809 (660bp, 730bp) for and Zadber-5 UBC-894 (910 bp) for Zadber-8 generated a unique DNA fragment by ISSR primer. These unique fragments can be used as a marker for identification of these cultivars, which will be useful for future conservation, maintenance and breeding programme. These accessions can also be used for developing the core collection of Zadber germplasm. UPGMA dendrogram divided all the accessions into two main clusters based on morpho-metric data. Zadber-7 was the most distinct from rest of the clusters. Zadber-2 and Zadber-4 were grouped together owing to their similarity in fruit morphology. 2-D plot showed three groups which was found less similar to the clustering pattern of UPGMA dendrogram. In 2-D plot, accession, Zadber-7 formed separate group but in the dendrogram it was grouped along with Zadber-2, Zadber-4, Zadber-5, Zadber-6 and Zadber-9. The high level of polymorphisms, in spite of the high morphological variability, could be explained by the fact that somatic mutations may be one of the sources of variability in Zadber. In the present study, ISSR markers proved to be useful for germplasm characterization and diversity analysis in *Z. nummularia* accessions. These results can be further used to manipulate genetic determinants of horticulturally important traits and to characterize the basis of productivity of Zadber accessions in India.

CONCLUSION

Findings of the present study revealed that 10 zadber germplasm were moderate to high diversity based on molecular and morphological assessment approaches. The results obtained will serve as a guide for germplasm management and crop improvement programmes. Significant variability was observed in the selected Zadber accessions by the analysis of five quantitative and 25 qualitative morphological characters of leaves, fruits and seeds which contributes to effective conservation and utilization of the zadber genetic resources. Molecular markers (ISSR) also showed high level of polymorphism (86.58%) and Jaccard's genetic similarity value of ISSR suggested moderate level of genetic diversity within the Zadber group. The present study revealed that morphological and molecular markers can be successfully utilized for determining genetic diversity and genetic relationship of Zadber cultivars and used in breeding programmes.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATION USED

ISSR: Inter Simple Sequence Repeats; **UPGMA:** Unweighed Pair Group Method with Arithmetic average; **PCA:** Principal Component Analysis; **CTAB:** Cetyl Tri-methyl Ammonium Bromide.

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GRAPHICAL ABSTRACT

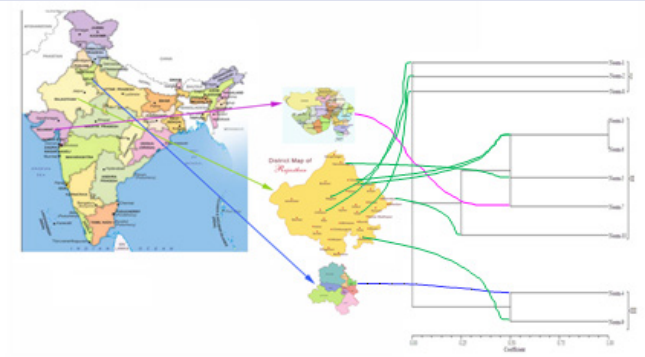

Figure. Showing the genetic diversity at the DNA characterization by ISSR molecular marker

HIGHLIGHTS OF PAPER

- Ten Zadber accessions collected from different agroclimatic regions of India, were evaluated for phenotypic and genotypic variations using morphological and inter-simple sequence repeats (ISSR) markers.
- Significant variability was observed in the selected Zadber accessions by the analysis of five quantitative and 25 qualitative morphological characters of leaves, fruits and seeds.
- ISSR markers also showed high level of polymorphism (86.58%). Jaccard's genetic similarity value of ISSR was found in the range of 0.45-0.77 (average 0.61) suggesting moderate level of genetic diversity.
- Two of the eleven ISSR primers were also able to generate cultivar specific amplicons, which may be used for identification of accessions Zadber-5 and Zadber-8.

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