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Existence of genetically diverse ecotypes of *Ziziphus nummularia*: a wild species of *ber* from western India

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ABSTRACT

***Ziziphus nummularia*, a multipurpose under-exploited, wild fruit tree of the arid region, tolerates various abiotic stresses. To improve the genetic resource of *Z. nummularia* and its sustainable usage, conservation and evaluation of this species is essential. Populations of *Z. nummularia* from hyper-arid (Jaisalmer), arid (Bikaner), and semi-arid (Godhra) regions of western India were collected and established in the field gene bank at ICAR-CIAH as baseline approach for conservation. Larger leaf, fruit and seed size, lesser number of leaf hairs were observed in *Z. nummularia* population from semi-arid in comparison to arid and hyper-arid regions. Genomic DNA of these populations was tested with 80 RAPD and 18 ISSR primers. Out of these primers, 26 RAPD and 14 ISSR primers were polymorphic. Phylogenetic analysis showed that populations of *Z. nummularia* from hyper-arid, arid and semi-arid region formed different clusters. The estimated gene flow value (0.697), diversity among populations (0.418), and Fst value (0.419) demonstrated that *Z. nummularia* has high genetic diversity within the population with limited gene flow between populations suggesting that different ecotypes of *Z. nummularia* exist in the arid and semi-arid regions.**

Key words: *Jharberi*, arid, abiotic stress, DNA markers, phylogenetic analysis.

INTRODUCTION

Ziziphus nummularia (Burm.f) Wight & Arn. commonly called "*jharberi*" (synonyms: *jjadiaber, birar, kokni-ber, bhor, zariab*) is a multipurpose, under-exploited, wild fruit tree species. It is distributed from Iran to India generally at altitudes up to 600m. In India, it is well distributed in dry and hot climate prevailing in arid and semi-arid regions of western plains, central India and extending to southern peninsular region. It is a bushy shrub, thorny, about 1.8-2.4 m height, leaves on short petiole, flowers 10-20 in auxiliary bundles, drupe globose of 8.3 mm diameter, red, glabrous (Pandey *et al.*, 7). It grows in the wild and valued for edible fruits, leaves as fodder, branches for fencing, wood as fuel, for construction and furniture, as folk medicine and role in soil conservation. *Z. nummularia* can tolerate various stresses like drought, salinity and temperature (Pareek, 8). It provides sustenance for the desert living animals and human population at the time of scarcity or during off-season. This species is very important in providing nutrition to desert dwellers during crop failure or famine. Therefore, *Z. nummularia* has vital role in the sustainability of desert ecosystem. The natural variations in *Z. nummularia* due to cross pollination, heterozygosity, and difference in ploidy level provide us opportunities for selection of better genotypes for various uses (Vashishtha, 14). To select these variations and promote sustainable conservation of this species, understanding its genetic diversity is far most important. Diversity among *Z. nummularia* genotypes at molecular level has not been studied so far. *Z. nummularia* is growing well even in the hot arid regions receiving average annual rainfall even lesser than 100mm and with extremes of temperature range between -2ºC and 50ºC (Awasthi and More, 2). Therefore, it is essential to collect and conserve the natural variations that are available in this species to exploit them for sustainable usage. Keeping these in view, the present study aimed (i) to explore the genetic variations of *Z. nummularia* at morphological and molecular level and (ii) to estimate gene flow among the different populations of *Z. nummularia* which are important for improvement and understanding of their evolution in the arid ecosystem.

MATERIALS AND METHODS

Surveys were conducted in the hyper-arid (Jaisalmer) (aridity index <0.03), arid (Bikaner) (aridity index 0.03-0.2) and semi-arid (Godhra) (aridity index 0.2-0.5) regions of India (Table 1 & 2) during November 2011. Matured red fruits of *Z. nummularia* were collected from 125 trees from each region which consists of five locations. Twenty five trees were selected from each location and their fruits were collected which represent a location. The seeds were extracted and seedlings were established (Pareek, 8) in the field gene bank of ICAR-CIAH. Data were

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Table 1. Places of sample collection and weather data.

Collection of sample (Place/ State)	Latitude and longitude	Range of average annual rainfall (mm)	RH range (%)	Average maximum temperature (ºC)	Average minimum temperature (ºC)
Jaisalmer/ Rajasthan	26º55'N & 70º54'E	110-209	26-84	23.8-43.5	4.1-26.7
Bikianer/ Rajasthan	28º01'N & 73º22'E	260-440	34-86	22.0-43.2	6.1-29.6
Godhra/ Gujarat	22º77'N & 73º60'E	839-1179	42-86	26.0-39.4	9.6-34.4

Table 2. Origin of *Z. nummularia* samples.

S. No.	Species/ cultivar	Sample ID	Collection of sample (Place or Village/ District/ State)
1	*Ziziphus nummularia*	CIAH-Zn-J1	Dholiya/ Jaisalmer/ Rajasthan
2	*Z. nummularia*	CIAH-Zn-J2	Chandan/ Jaisalmer/ Rajasthan
3	*Z. nummularia*	CIAH-Zn-J3	Ramgarh/ Jaisalmer/ Rajasthan
4	*Z. nummularia*	CIAH-Zn-J4	Tanot/ Jaisalmer/ Rajasthan
5	*Z. nummularia*	CIAH-Zn-J5	Devikot/ Jaisalmer/ Rajasthan
6	*Z. nummularia*	CIAH-Zn-B1	Deshnok/ Bikaner/ Rajasthan
7	*Z. nummularia*	CIAH-Zn-B2	Nokha/ Bikaner/ Rajasthan
8	*Z. nummularia*	CIAH-Zn-B3	Raisar/ Bikaner/ Rajasthan
9	*Z. nummularia*	CIAH-Zn-B4	Nal/ Bikaner/ Rajasthan
10	*Z. nummularia*	CIAH-Zn-B5	Beechwal/ Bikaner/ Rajasthan
11	*Z. nummularia*	CIAH-Zn-G1	Vejalpur/ Godhra/ Gujarat
12	*Z. nummularia*	CIAH-Zn-G2	Pavagarh/ Godhra/ Gujarat
13	*Z. nummularia*	CIAH-Zn-G3	Shehera/ Godhra/ Gujarat
14	*Z. nummularia*	CIAH-Zn-G4	Kalol/ Godhra l/ Gujarat
15	*Z. nummularia*	CIAH-Zn-G5	Tuwa/ Godhra/ Gujarat

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recorded on trees of *Z. nummularia* during survey on mother trees and five year old trees in the field gene bank. Morphological characters (Table 3) such as tree shape, leaf size and shape, number of hairs on adaxial and abaxial surface (6.28 mm^2) counted microscopically, thorn length, fruit size and shape, number of seeds per stone and seed viability were recorded on these three populations.

Genomic DNA was extracted from 100 mg of young emerging leaves of *Z. nummularia* in the field gene bank from each sample separately by CTAB method (Doyle & Doyle, 4) without liquid nitrogen (Sharma *et al.*, 10). Eighty random decamer primers belonging to the series of OPBE, OPBA, OPA & OPN (Operon Technologies, USA) were used. PCR protocol for RAPD and ISSR primers as described by Khan *et al*. (5) was followed. All the amplified bands were counted manually along with their size. The presence of band was scored as '1' and absence as '0'. A pair-wise matrix of genetic distances between genotypes was determined using Jaccard similarity coefficient and a phylogenetic tree based on UPGMA was constructed using NTSYSpc-2.02e version 2.0.1.5 software (Applied Biostatistics Inc, NY, USA). Estimation of genetic variation on different parameters was carried out by using the POPGENE software version 1.32 (Yeh *et al.*, 16) Gene flow (Nm) was estimated using the formula Nm=0.5(1-Gst)/Gst.

RESULTS AND DISCUSSION

It was demonstrated for the first time that populations of *Z. nummularia* in India are genetically more diverse and formed different ecotypes (Fig. 3) according to the region with higher adaptability to various abiotic stresses mainly drought coupled with high temperature. Among three populations, *Z. nummularia* from Jaisalmer is hardy to various abiotic stresses and are well distributed in the hyper arid region where annual rainfall is as little as 80 mm and aridity index is lower than 0.03(Sharma and Tiwari, 12). It could be due to its deep rooting behaviour, leaf rolling upon stress, withholding water for longer duration, greater membrane stability, lesser stomatal conductance coupled with increase in

Table 3. Morphological differences among three populations of *Z. nummularia* occurring in western India.

Character	*Z. nummularia*-Jaisalmer	*Z. nummularia*-Bikaner	*Z. nummularia*-Godhra
Spreading nature of tree	Mostly bushy	Mostly bushy	Bushy
Leaf shape and size (Length cm × Width cm)*	Mostly round to elliptical (1.45 ± 0.16 × 1.17 ± 0.14)	Mostly round to elliptical (1.45 ± 0.11 × 1.29 ± 0.17)	Mostly round (2.3 ± 0.19 × 2.0 ± 0.23)
Number of leaf hairs in abaxial surface (6.28 mm^2)*	7898.4 ± 631.91	7523.8 ± 591.48	4198.7 ± 461.53
Number of leaf hairs in adaxial surface (6.28 mm^2)*	210 ± 14.47	205.6 ± 25.54	63.6 ± 6.60
Length of straight thorn (mm)*	14.3 ± 1.10	14 ± 1.54	11.1 ± 0.94
Fruit shape and diameter (cm)*	Round, 0.93 ± 0.08	Round, 1.09 ± 0.07	Round, 1.30 ± 0.07
Stone shape and number of seeds per stone	Round and contains mostly 1 seed (range 84.0-90.6%)	Round and contains 1-2 seeds. (one seed-range 61.7 to 72.2%)	Round and contains 2 seeds
Seed viability (%)*	93.3 ± 1.0	88.7 ± 1.0	92.7 ± 1.2

'*' - Value ± standard deviation

reducing sugars, proline and catalase during drought period than other two populations (Sivalingam *et al.*, 13). Populations from Jaisalmer also showed higher regeneration capacity upon receiving water immediately after long dry spell. Comparison of three populations of *Ziziphus nummularia* indicated that the significant difference on morphological parameters was observed among these populations (Fig. 1 & Table 3). *Z. nummularia* from Jaisalmer and Bikaner regions were bushier in nature, has increased pubescence on leaf surfaces, smaller fruits and stone contained predominantly one seed compared to *Z. nummularia* from Godhra which had mostly two seeds per stone and were comparatively bigger in size. However, much difference was not observed for viability of seeds among three populations (88 to 93%). The leaf size of Godhra population was bigger and round in shape than other two populations. No variation was observed in nature of thorns among the three populations. All populations had two thorns; one long and straight and another short and curved. However, the length of straight thorns was longer (14 mm) in populations from Jaisalmer and Bikaner than Godhra (11 mm). Morphological features of Jaisalmer populations such as small leaf and fruit, increased pubescence on both surfaces of leaf and bushy nature of tree could be the mechanisms of its adaptation and survival in the hyper arid ecosystems. The populations of *Z. nummularia* have been conserved in field gene bank to protect it from threat and identify genetic changes that have occurred during evolution. Out of 80 RAPD primers, twenty six were found polymorphic among three populations. Totally 232 loci were amplified by these RAPD primers and 206 were found to be polymorphic. The polymorphism detected by these primers ranged between 33.3 % and 100 % with average polymorphism of 86.97 % and polymorphic information content (PIC) of 0.42. Primers such as OPBE08, OPBE20, OPA05, OPA17, OPA19, OPA20, OPN05, OPN07, OPN09 scored a PIC of near maximum value (0.5). Out of 18 ISSR primers, 14 were polymorphic (69.07 %) and the range of polymorphism was between 25 % and 100 %. The average PIC was found to be 0.4. The details of the size of the bands, number of loci scored and polymorphic loci and PIC of individual primers are given in Table 4 and Fig. 1. Comparison between populations of *Z. nummularia* from Jaisalmer and Bikaner had maximum average Jaccard's similarity co-efficient of 0.74 followed by Bikaner and Godhra (0.69), and the least was between Jaisalmer and Godhra (0.65). Cluster analysis revealed that all three populations formed separate clusters (Figs. 2 & 3). The range of observed and effective number of alleles in the populations of *Z. nummularia* was 1.436-1.598 and 1.322-1.369, respectively. The genetic variation of *Z. nummularia* within subpopulations (58.1%) and among subpopulations (41.9%) is an indicator of the existence of wide genetic variation in *Z. nummularia* in the western parts of India. The estimated Nei's gene diversity (*h*) and Shannon's Information index (*I*) range was 0.178-0.216 with an overall diversity of 0.334 and 0.258- 0.323 with an average value of 0.495, respectively. These data revealed that the population from the Jaisalmer region had higher genetic variation compared to populations of Bikaner and Godhra. Estimated percentage of polymorphic loci was in the range of 43.57-59.75 with relatively

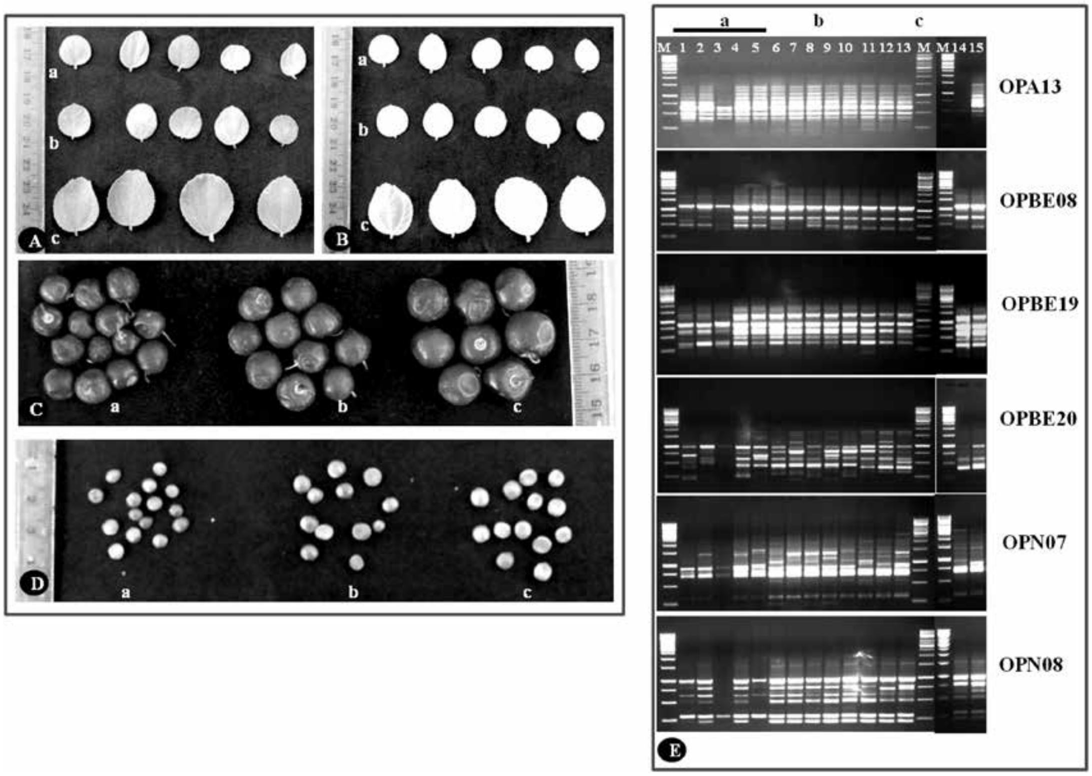

Fig. 1. Morphological and molecular diversity among three populations of *Z. nummularia.* (A) adaxial surface of leaf (B) abaxial surface of leaf (C) fruits (D) seeds and (E) RAPD profile; a- *Z. nummularia*-Jaisalmer; b- *Z. nummularia*-Bikaner; c- *Z. nummularia*-Godhra Lanes M-1Kbp DNA ladder; 1 to 5 -*Z. nummularia*-Jaisalmer; 6 to 10 - *Z. nummularia*-Bikaner; 11 to 15 - *Z. nummularia*-Godhra.

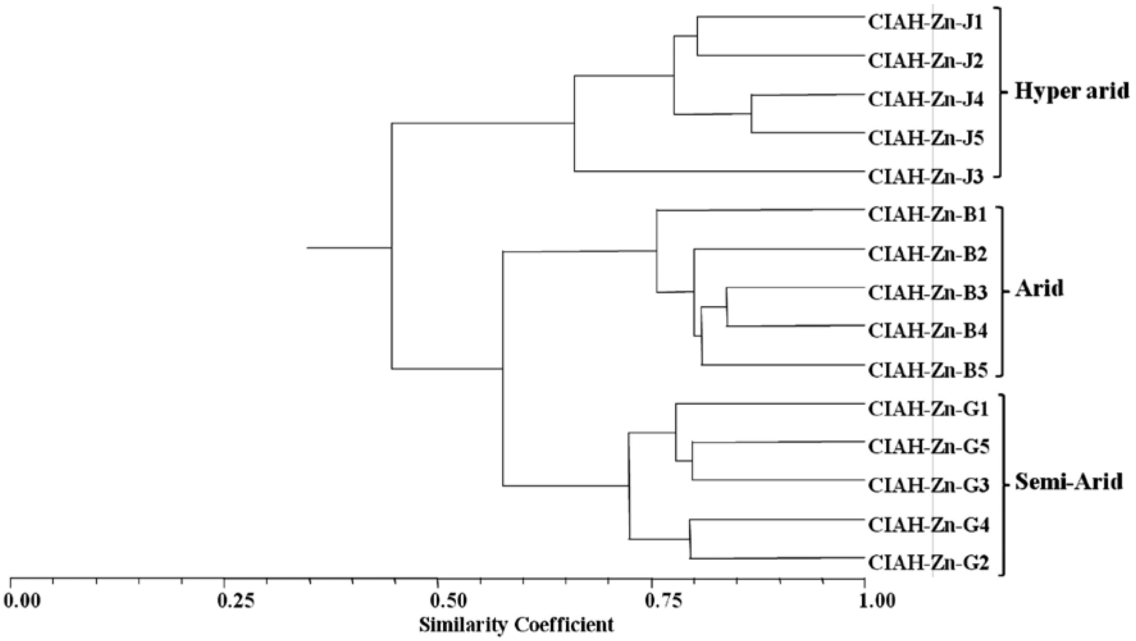

Fig. 2. Dendrogram based on different *Z. nummularia* populations from western region of India revealed by RAPD and ISSR markers. Right brace indicates clusters. The populations of *Z. nummularia* grouped according to their geographical locations of their origin.

Table 4. Details of RAPD and ISSR primers used in this study and their polymorphism.

S. No.	Primer name	Sequence (5' to 3')	Band size range (bp)	Loci scored	No. of polymorphic loci	% Polymorphism	PIC
RAPD							
1	OPBE04	CCCAAGCGAA	250-1300	11	11	100.00	0.48
2	OPBE08	GGGAAGCGTC	400-1100	8	7	87.50	0.49
3	OPBE11	GTCCTGCTGT	300-2000	10	9	90.00	0.47
4	OPBE12	GGTTGTTCCC	300-1400	7	7	100.00	0.48
5	OPBE16	CTCCACGACT	300-1350	8	8	100.00	0.35
6	OPBE17	GGGAAAAGCC	500-1200	4	2	50.00	0.28
7	OPBE19	AGGCCAACAG	300-1300	6	3	50.00	0.22
8	OPBE20	CAAAGGCGTG	200-1700	11	10	90.91	0.49
9	OPA02	TGCCGAGCTG	300-1200	6	2	33.33	0.16
10	OPA04	AATCGGGCTG	150-2100	9	6	66.67	0.33
11	OPA05	AGGGGTCTTG	250-2100	16	16	100.00	0.49
12	OPA06	GGTCCCTGAC	450-900	5	5	100.00	0.48
13	OPA08	GTGACGTAGG	100-2000	12	11	91.67	0.47
14	OPA11	CAATCGCCGT	100-1300	10	9	90.00	0.48
15	OPA13	CAGCACCCAC	200-2000	12	11	91.67	0.31
16	OPA16	AGCCAGCGAA	200-1600	11	9	81.82	0.44
17	OPA17	GACCGCTTGT	300-1500	10	10	100.00	0.49
18	OPA19	CAAACGTCGG	100-1300	7	6	85.71	0.49
19	OPA20	GTTGCGATCC	200-1500	6	6	100.00	0.49
20	OPN04	GACCGACCCA	750-1300	7	7	100.00	0.31
21	OPN05	ACTGAACGCC	300-1600	8	8	100.00	0.49
22	OPN06	GAGACGCACA	100-1500	11	9	81.82	0.47
23	OPN07	CAGCCCAGAG	250-2500	13	12	92.31	0.49
24	OPN08	ACCTCAGCTC	200-1500	9	7	77.78	0.35
25	OPN09	TGCCGGCTTG	250-1400	7	7	100.00	0.49
26	OPN10	ACAACTGGGG	100-1800	8	8	100.00	0.43
			Average =	8.92	7.92	86.97	0.42
ISSR							
Primer name[a]	UBC[b] code	Sequence (5`–3`)	Band size range (bp)	Loci scored	No. of polymorphic loci	% Polymorphism	PIC
P2	809	AGAGAGAGAGAGAGAGG	500-2000	7	4	57.14	0.41
P3	814	CTCTCTCTCTCTCTCTA	400-850	4	3	75.00	0.42
P4	825	ACACACACACACACACT	750-1700	7	4	57.14	0.47
P5	829	TGTGTGTGTGTGTGTGC	400-1600	8	5	62.50	0.35
P6	240	GAGAGAGAGAGAGAGACTT	800-1500	4	1	25.00	0.30
P8	848	CACACACACACACACAAGG	1400-1700	3	3	100.00	0.44
P9	850	GTGTGTGTGTGTGTGTCTC	900-3000	4	3	75.00	0.40
P10	854	TCTCTCTCTCTCTCTCAGG	600-1500	4	4	100.00	0.42
P11	855	ACACACACACACACACCTT	800-2000	5	2	40.00	0.40
P12	856	ACACACACACACACACCTA	700-2000	7	4	57.14	0.40
P13	876	GATAGATAGACAGACA	200	1	1	100.00	0.32
P14	880	GGAGAGGAGAGGAGA	750-1500	3	3	100.00	0.44
P15	889	AGTCGTAGTACACACACACACAC	300-2100	8	5	62.50	0.42
P16	890	ACGACTACGGTGTGTGTTTGTGT	500-3000	9	5	55.56	0.41
			Average =	5.28	3.36	69.07	0.40

[a] -Primer name used in this study; b- University of British Columbia. PIC- Polymorphic Information Content.

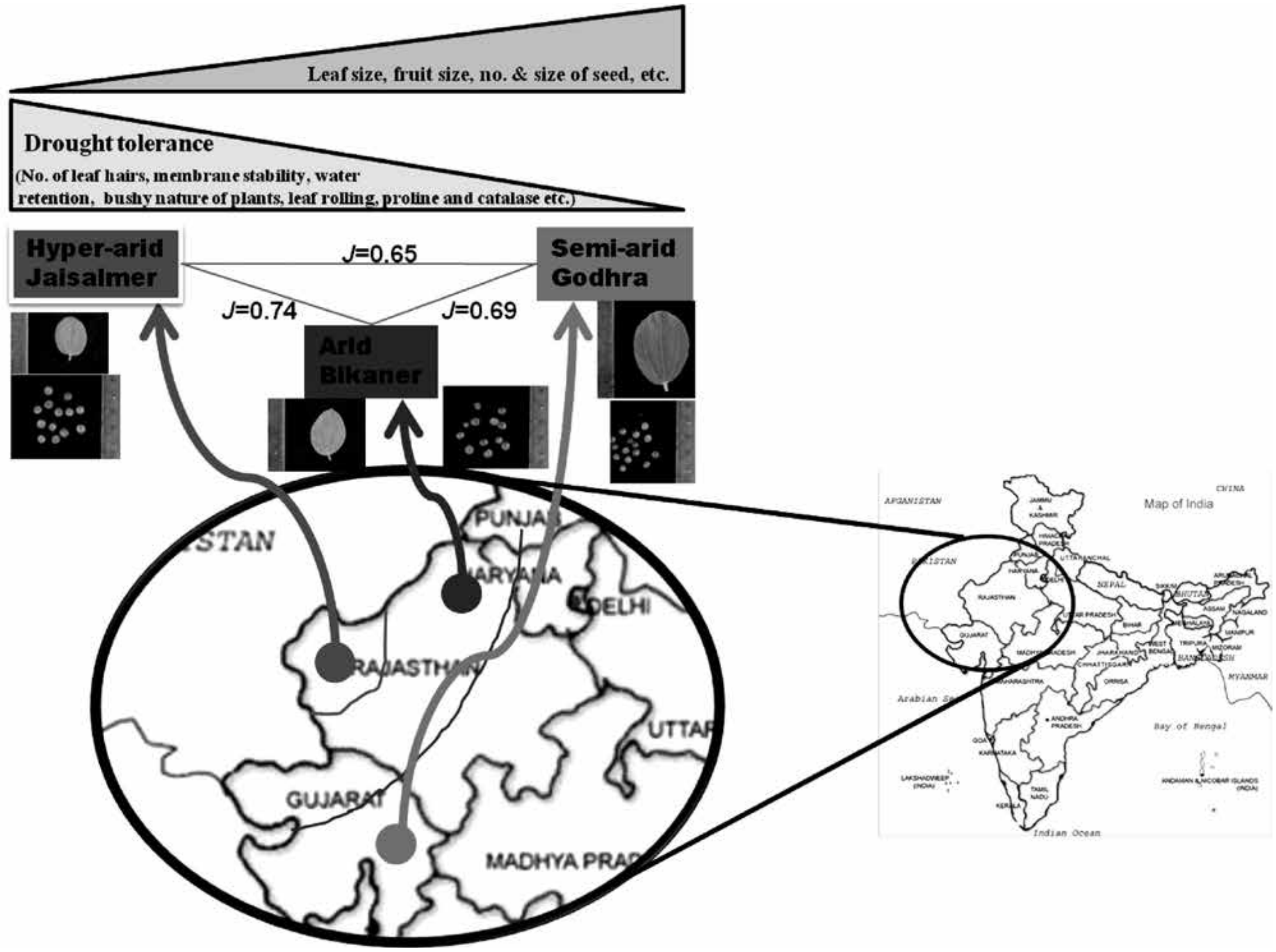

Fig. 3. Different ecotypes of *Z. nummularia* in the western region of India which is enlarged from map of India shown in dark circle with name of the states with border. Photographs showing morphology of leaf and seeds of three populations have shown with the respective population of *Z. nummularia.* Dark colour arrow lines indicate the region of sample collection. *J*- Average Jaccord's similarity co-efficient between populations.

high mean value of 91.29. The diversity among the populations (0.418), gene flow value (0.697) and Fst value (0.419) indicated the existence of a large genetic heterogeneity within a population and lesser gene flow between populations of *Z. nummularia* (Table 5). This genetic variation and large population of this species increases its adaptive response to local selection (Aitken & Whitlock, 1). The genetic diversity reflected in the populations of Jaisalmer at DNA level may be due to possible heterozygosity of genome and also plasticity of these genomes to adapt to the extreme environmental conditions of the arid region (Sharma *et al.*, 11). It is possible that the evolution of *Ziziphus* or ziziphoid group is reasonably older (~ 95 million years ago) (Richardson *et al.*, 9) than the expansion of arid environment in the western region of India, now called the Thar Desert. The aridification in this region might be recent i.e., ~0.6-1.6 million years ago. Before aridification, conditions might have been akin to semi-aridity. *Z. nummularia* could have been well distributed in present day Thar Desert as one of the semi-arid plants. During aridification, *Z. nummularia* plants possessing allelic or gene frequencies tolerant to aridification may have been selected under intense natural selection pressure (Aitken & Whitlock, 1). Over time, due to natural selection, all three types could have diverged from common population adapting to particular ecosystem; thereby, forming a different ecotype or ecoclines of *Z. nummularia* (Fig. 2) (Kremer *et al.*, 6). The reduced gene flow in *Z. nummularia* may be due to elimination of alleles entering into locally adapted new population by selection. Stronger the selection, the more rapidly immigrant alleles of lower fitness will be eliminated from the population; thereby, reducing effective migration rates and increasing the time to

Table 5. Genetic diversity static and differentiation parameters for three populations of *Z. nummularia* revealed by RAPD and ISSR.

Population	Ss	Na ± SD	Ne ± SD	*h* ± SD	*I* ± SD	Pp (%)	Np	Gst	Nm	Ht	Hs
Jaisalmer	5	1.598 ± 0.491	1.369 ± 0.374	0.216 ± 0.199	0.323 ± 0.285	59.75	144				
Bikaner	5	1.436 ± 0.497	1.322 ± 0.410	0.178 ± 0.215	0.258 ± 0.306	43.57	105				
Godhra	5	1.444 ± 0.498	1.350 ± 0.430	0.189 ± 0.223	0.271 ± 0.315	44.40	107				
	15	-	-	0.334 ± 0.158	0.495 ± 0.210	91.29	220	0.418	0.697	0.334 ± 0.025	0.194 ± 0.018

Ss = Sample size; *h* = Nei's (1973) gene diversity; *I* = Shannon's Information index (Lewontin, 1972); Na = Observed number of alleles; Ne = Effective number of alleles (Kimura and Crow, 1964); Pp = Percentage of polymorphic loci; Np = Number of polymorphic loci; Gst = Diversity among populations; Nm = gene flow 0.5 (1 - Gst)/Gst; Ht = overall genetic Diversity; Hs = average subpopulation genetic diversity; SD = Standard deviation

coalescence (Charlesworth *et al.*, 3). Population of *Z. nummularia* occurring in the semi-arid region may be a natural group. However, *Z. nummularia* populations prevailing in the arid region or Thar Desert are not part of a natural group and have adapted to arid environment with highest relative fitness (Wright & Gaut, 15).

These genotypes of Z. *nummularia* in the arid ecosystem must be conserved for its improvement by (i) Isolation of novel genes particularly involved in abiotic stresses; (ii) Enhancing local adaptation of semi-arid genotypes by assisted gene flow in accordance with climate change; and (iii) Identification and isolation of candidate genomic region /candidate loci as influenced by selection pressure will be useful in increasing adaptation to the extreme environments of these species.

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REFERENCES

1. Aitken, S.N. and Whitlock, M.C. 2013. Assisted gene flow to facilitate local adaptation to climate change. *Annu. Rev. Ecol. Evol. Syst.* **44**: 367-88.
2. Awasthi, O.P. and More, T.A. 2009. Genetic diversity and status of *Ziziphus* in India. *Acta. Hort.* **840**: 33-40.
3. Charlesworth, B., Nordborg, M. and Charlesworth, D. 1997. The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided inbreeding and out crossing populations. *Genet. Res.* **70**: 155-74.
4. Doyle, J.J. and Doyle, J.L. 1990. Isolation of plant DNA from fresh tissue. *Focus*, **12**: 13-15.
5. Khan, H., Sivalingam, P.N., Chauhan, S., Awasthi, O.P. and More, T.A. 2013. Improved crossing technique and identification of true F1 hybrids of *Ziziphus mauritiana* Lam. by molecular markers. *Sci. Hortic.* **150**: 164-71.

6. Kremer, A., Potts, B.D. and Delzon, S. 2014. Genetic divergence in forest trees: understanding the consequences of climate change. *Funct. Ecol.* **28**: 22-36.

7. Pandey, A., Singh, R., Radhamani, J. and Bhandari, D.C. 2010. Exploring the potential of *Ziziphus nummularia* (Burm f) Wight et Arn from drier regions of India. *Genet. Resour. Crop Ev.* **57**: 929-36.

8. Pareek, O.P. 2001. Ber. International Centre for Underutilized Crops Southampton UK, p. 290.

9. Richardson, J.E., Fay, M.F., Cronk, Q.C.B. Bowman, D. and Chase, M.W. 2000. A phylogenetic analysis of rhamnaceae using *RBCL* and *TRNL-F* plastid DNA sequences. *Am. J. Bot.* **87**: 1309-24.

10. Sharma, R., Mahla, H.K., Mohapatra, T., Bhargava, S.C. and Sharma, M.M. 2003. Isolating plant genomic DNA without liquid nitrogen. *Plant. Mol. Biol. Rep.* **21**: 43-50.

11. Sharma, S.K., Kumar, S., Rawat, D., Kumaria, S., Kumar, A. and Rao, S.R. 2011. Genetic diversity and gene flow estimation in *Prosopis cineraria* (L.) Druce: A key stone tree species of Indian Thar Desert. *Biochem. Sys. Ecol.* **39**: 9-13.

12. Sharma, A.K. and Tiwari, J.C. 2005. Arid zone forestry with special reference to Indian hot arid zone. In: Vol-II, Forests and Forest Plants. Encyclopaedia of life support systems (EOLSS). EOLSS publishers, Oxford, UK.

13. Sivalingam, P.N., Singh, D., Bhargava, R. and Sharma, S.K. 2014. CIAHZN-J- A low moisture-stress tolerant *Ziziphus nummularia* from Jaisalmer. *ICAR News* **20**: 10.

14. Vashishtha, B.B. 2001. *Ber* varieties a monograph, Agrobios (India), Jodhpur.

15. Wright, S.I. and Gaut, B.S. 2005. Molecular population genetics and the search for adaptive evolution in plants. *Mol. Biol. Evol.* **22**: 506-19.

16. Yeh, F.C., Young, R.C. and Boyle, T. 1999. Microsoft Window-based Freeware for Population Genetic Analysis (POPGENE Ver 1.32) ftp://ftpmicrosoftcom/softlib/mslfiles/hpglexe

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