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Revista de Biología Tropical, vol. 61, núm. 1, marzo, 2013, pp. 311-320
Universidad de Costa Rica
San Pedro de Montes de Oca, Costa Rica

Revista de Biología Tropical,
ISSN (Printed Version): 0034-7744
rbt@cariari.ucr.ac.cr
Universidad de Costa Rica
Costa Rica

Combining molecular-marker and chemical analysis of *Capparis decidua* (Capparaceae) in the Thar Desert of Western Rajasthan (India)

Sushil Kumar[1,2], Ramavtar Sharma[1],Vinod Kumar[2], Govind K. Vyas[3] & Abhishek Rathore[4]

1. Plant Biotechnology Centre, SK Rajasthan Agricultural University, Bikaner 334006, India; ras_rau@rediffmail.com
2. NRC on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi-110012; veenu_yadav2003@rediffmail.com
3. Department of Botany, University of Rajasthan, Jaipur, 302004, India; govindvyas111@yahoo.co.in
4. International Crops Research Institute for Semi-Arid Tropics, Hyderabad, 502324, India; a.rathore@cgiar.org

Received 23-I-2012. Corrected 08-VIII-2012. Accepted 18-IX-2012.

Abstract: The Thar Desert, a very inhospitable place, accommodates only plant species that survive acute drought, unpredictable precipitation, and those can grow in the limited moisture of sandy soils. *Capparis decidua* is among one of the few plants able to grow well under these conditions. This species is highly exploited and has been naturally taken, as local people use it for various purposes like food, timber and fuel, although, no management or conservation efforts have been established. The present study was conducted in this arid area of Western Rajasthan (India) with the aim to obtain preliminary molecular information about this group of plants. We evaluated diversity among 46 samples of *C. decidua* using chemical parameters and random amplified polymorphic DNA (RAPD) markers. Fourteen chemical parameters and eight minerals (total 22 variables) of this species fruits were estimated. A total of 14 RAPD primers produced 235 band positions, of which 81.27% were polymorphic. Jaccard's similarity coefficients for RAPD primers ranged from 0.34 to 0.86 with a mean genetic similarity of 0.50. As per observed coefficient of variation, NDF (*Neutral Detergent Fiber)* content was found to be the most variable trait followed by starch and soluble carbohydrate. The Manhattan dissimilarity coefficient values for chemical parameters ranged between 0.02-0.31 with an average of 0.092. The present study revealed a very low correlation (0.01) between chemical parameters and RAPD-based matrices. The low correlation between chemical- and RAPD-based matrices indicated that the two methods were different and highly variable. The chemical-based diversity will assist in selection of nutritionally rich samples for medicinal purpose, while genetic diversity to face natural challenges and find sustainable ways to promote conservation for future use. Rev. Biol. Trop. 61 (1): 311-320. Epub 2013 March 01.

Key words: *Capparis decidua*, chemical diversity, nutritional parameters, desert, RAPD.

Capparis decidua (Forssk.) Edgew., commonly known as ker, a rangeland perennial bushy shrub with spines, belongs to *Capparaceae* which is found principally in tropical and warm temperate regions. It is mostly found in India, Pakistan, Niger, Nigeria, Senegal Tibesti (West Chad), much of the Sudan (except the extreme South) the Arabian Peninsula, Jordan, Iran, the Mascarene Islands and Natal. *Capparaceae* comprises *circa* 650 species of small trees, of which 26 species are reported to occur in India. Members of this family contain thioglucosides which release isothiocyanates ("mustard oils") when the plants are damaged. Typically, the plants yield methyl isothiocyanate from methyl glucosinolate, otherwise known as glucocapparin. These mustard oils have skin irritant activity and may also have contact allergenic activity (Mitchell & Jordan 1974, Richter 1980).

Some species of capers are known to be edible and *C. decidua* is one among them. *C. decidua* is 4-5m in height, or occasionally a small tree with apparently leafless branches, hanging in bundles. Leaves are very minute (2mm long), with a very short life span on young shoots, this way, the plant looks leafless most of the time. Flowers of this xeric underutilized shrub are pink, red-veined, in small groups along the leafless shoots, in the axils of the spines. The flower buds of this spiny tree are cooked as a potherb, and also pickled (Jacobs 1965). The fruit is a small many-seeded ovoid or subglobulous, slightly mucronate pink berry of the size and shape of a cherry, becoming blackish when dry and eaten by birds. The seeds contain glucocapparin (Juneja *et al.* 1971) from which the mustard oil methyl isothiocyanate is released when the plant material is crushed. It coppices well and produces root suckers freely. The flower buds called capers or fruits collected from *C. decidua* are pickled and used as a condiment. As an estimate, *C. decidua* is distributed over 3 450km^2 plains in Nagaur, Bikaner and Jodhpur districts of Rajasthan with an estimated annual production of 7 000 tonnes of fruits.

In alkaline and sandy soil of piedmont plains, eroded rocky surface and gravelly plain, *C. decidua* is dominant among the community of desert plant species. It is extremely drought-resistant and tolerates some frost, resulting in an interesting plant because of its excellent adaptation to arid conditions. It can also tolerate fire and termites. It can be found at the altitude range from 300-1 200m with mean annual rainfall of 100-750mm and mean annual temperature of 25-41°C. It has been found to be one of the best species for afforestation, reforestation and shelter belts to check the movement of sand in the Thar Desert, India (Pandey & Rokad 1992). Medicinally, young roots of the plants are applied to cure boils and swelling, the bark is said to be useful in asthma. The very bitter roots are used in the Indian and Farsi pharmacopoeia. The shoots and young leaves contain a rubefacient and vesicant principle (Chopra & Badhwar 1940, Behl *et al.* 1966). Local people eat the fruit to reduce blood sugar, the plant extract for eczema and the decoction of the plant is taken orally in rheumatism.

Being an important sand binder in sand dunes of the desert, the overexploitation of so long standing *C. decidua* plants for mankind purposes would surely change those ecosystems. Plant area is day by day shrinking to some isolated patches in the Thar Desert of Rajasthan. Quick conservation of biodiversity is urgently required to protect important plant species like *C. decidua*. For the efficient utilization of plant genetic resource collections, information on genetic diversity and the relationships within the species is essential. The diversity knowledge available for economic traits is the key for improvement and/or domestication of any species. The information recorded for various morphological traits in *C. decidua* has been generated by various workers sporadically but no systematic information is available for its nutritional value. While, domestication is urgently needed to preserve the species and put it to economic use in an area of adaptation; information regarding nutritional value is of utmost importance to select the desired types and also important for the information regarding diversity at a molecular level. Limited work on *C. decidua* is available for its diversification through chemical/nutritional and molecular parameters which are important in presenting its nutritional value and diversity level (Kumar *et al*. 2011). So, the present study was aimed towards the assessment of diversity of *C. decidua* plants from a densely populated location of the Thar Desert of Western Rajasthan using chemical/nutritional and molecular marker. The diversity for chemical and nutritional properties will improve the bioavailability of micronutrients, and their modification by various processing techniques. Such information would be of fundamental importance in addressing dietary deficiencies in impoverished rural communities. New generation molecular markers (SSR, SNP) are not developed for this species, so RAPD was selected for the diversity analysis. RAPD is preferred over other random markers due to its simplicity, speed and relatively low cost. Being a fast and sensitive method, RAPD can be quickly and efficiently applied to identify useful polymorphisms.

Moreover, it is successfully used to analyze diversity and genetic structure of wild plant species (Xu *et al.* 2003, Zhang *et al.* 2005, Vyas *et al.* 2009).

MATERIALS AND METHODS

Plant materials: In the present study, 46 wild samples of *C. decidua* fruits were collected randomly from a single population covering an area of 5km^2 of Nagaur (North-East) region of Rajasthan (India). Nagaur District is situated between 260.25'-270.40' N and 730.10'-750.15' E. Its geographical spread is a good combination of plain, hills, sand mounds and as such it is a part of the great Indian Thar Desert. The district of Nagaur is poor in forest resources. Scanty rainfall, sand dunes and deep water table constraints account for this. The maximum temperature recorded in district is 47°C with 0°C as the lowest recorded temperature. The average rainfall in the district is 36cm and 51.5% humidity. Scrub, xerophytic type habitat with *C. decidua*, *Prosopis cineraria* and *Acacia tortolis* are the dominant plants of this region.

Chemical and mineral analysis: A total of 22 parameters (14 chemical/8 mineral) were studied (Table 1). The fresh weight of fruits was taken in the field using battery operated weighing balance (Sartorius TE-153S-DS-MG, Germany); fruits were taken to the laboratory in different sample bottles, dried and analysed. Moisture content was obtained by heating the samples to a constant weight in a thermostatically controlled oven at 100°C (ICMR 1983). The ash, dietary fiber (hemicellulose, cellulose and lignin), crude fiber, crude protein, crude fat, total carbohydrate, vitamin C content and calcium contents were obtained using the methods described by Association of Official Analytical Chemists (AOAC 1995). Protein was

TABLE 1
Mean, coefficient of variance and range of different chemical parameter in 46 *C. decidua* fruits collected from Nagaur (Western Rajasthan)

Variables	Minimum	Maximum	Mean	SD	CV
Moisture (%)	63.7	75.01	69.66	2.62	5.69
Phosphorus (mg/100g)	179.33	273.82	219.05	17.52	38.08
Magnesium (mg/100g)	43.64	57.8	49.16	4.16	9.05
Iron (mg/100g)	1.3	9.33	4.64	1.66	3.61
Zinc (mg/100g)	0.02	0.79	0.31	0.18	0.4
Copper (mg/100g)	1.23	2.42	1.94	0.3	0.65
Sodium (mg/100g)	71.39	290.5	160.64	45.64	99.22
Calcium (%)	2.87	3.52	3.24	0.14	0.3
Crude protein (%)	11.88	20.04	14.94	2.1	4.58
Proline (mg/100g)	6.5	17.6	11.76	2.55	5.55
Total carbohydrate (%)	68.16	78.32	73.48	2.43	5.28
Soluble carbohydrate (%)	11.67	24.5	18.03	2.83	6.15
Starch (%)	9.23	21.76	15.28	3.15	6.85
Crude fiber (%)	7.6	16.5	10.94	2.42	5.25
NDF (%)	23.9	43	30.48	4.58	9.95
Hemicellulose (%)	8.6	15.8	11.45	2.1	4.57
Cellulose (%)	6.8	13	8.91	1.7	3.7
Lignin (%)	6.1	10.3	7.62	1.04	2.26
Crude fat (%)	3.2	7.9	5.38	1.01	2.19
Total ash (%)	5.3	6.8	5.97	0.37	0.8

SD = standard deviation, CV = coefficient of variation.

determined using the micro-Kjeldhal method. The soluble sugar and starch was estimated by the Anthrone method as suggested by Dubois *et al.* (1951). The mineral composition (magnesium, iron, phosphorus zinc, manganese, copper, and cobalt) was determined through the Atomic Absorption Spectrophotometer (Bishnoi & Brar 1988, AAS Model: GBC-932), while sodium was estimated using flame photometer (AOAC 1995, Sistronics). Finally, proline was estimated by a method given by Bates *et al*. (1973).

DNA isolation and PCR amplification: The method of Sharma *et al.* (2003) was used to extract total genomic DNA from tender twigs along with leaves of all the 46 samples. The DNA samples were treated with RNAase, assessed on 0.8% agarose gel and diluted to 25ng/µL for PCR amplification. A set of 14 random primers of OPF, OPG and OPH series (Operon Technologies Inc., Alameda, California) was selected for RAPD analysis (Table 2). The RAPD primers of OPF and OPG series were selected from study of Vyas *et al*. (2009). The PCR reactions were carried out in a 25µL reaction mixture containing 1X assay buffer, one unit of Taq DNA polymerase (Bangalore GeneiPvt. Ltd., India), 200µM of each dNTPs (Bangalore GeneiPvt. Ltd., India), 0.2µM primers and 50ng of template DNA in thermal cycler (Model-CGI-96, Corbett Research, Australia). The PCR reaction was performed in 45 cycles: one cycle of denaturation at 94°C for 4min followed by 44 cycles of denaturation at 94°C for 1min, primer annealing at 37°C for 1min and elongation at 72°C for 2min, followed by a final step of extension at 72°C for 4min. The PCR products were mixed with 10X DNA loading buffer and separated on 1.2% agarose gel containing 0.5µg/mL of ethidium bromide. For each RAPD primer, the presence (1) or absence (0) of bands in each accession was scored to generate rectangular data matrix (qualitative data matrix). The RAPD bands were scored for the presence (1) or absence (0) and each band that was regarded as a locus. Similarity matrix was constructed using the Jaccard's similarity coefficients and subjected to UPGMA (unweighted pair-group method with arithmetic averages) analysis to generate a dendrogram. The matrices derived from RAPD and chemical data were correlated using MXCOMP module of NTSYS pc. The discriminatory power of RAPD primers was analyzed using the method of Tessier *et al.* (1999).

TABLE 2
Primer sequences, total bands, polymorphic bands and discrimination power of RAPD primers used for *C. decidua* diversity analysis

Primers	Sequences (5'–3')	Total bands	Polymorphic bands	% polymorphism	Discrimination power
OPF1	ACGGATCCTG	15	13	86.67	0.75
OPF2	GAGGATCCCT	14	08	57.14	0.91
OPF5	CCGAATTCCC	20	19	95.00	0.85
OPF9	CCAAGCTTCC	16	16	100.00	0.97
OPF11	TTGGTACCCC	20	18	90.00	0.93
OPG4	AGCGTGTCTG	21	14	66.67	0.72
OPG6	GTGCCTAACC	10	08	80.00	0.77
OPG7	GAACCTGCGG	09	07	77.78	0.95
OPG11	TGCCCGTCGT	15	09	60.00	0.84
OPG8	TCACGTCCAC	19	16	84.21	0.69
OPG16	AGCGTCCTCC	21	18	85.71	0.79
OPH19	CTGACCAGCC	18	14	77.78	0.94
OPH20	GGGAGACATC	17	13	76.47	0.91
OPH21	ACTCCGCAGT	20	18	90.00	0.89

Manhattan distance coefficients were calculated for chemical/mineral parameters. UPGMA based dendrogram was constructed using Manhattan dissimilarity coefficients after the standardization of observations. All calculations were done using computer program NTSYSpc ver 2.02 (Rohlf 1998). Arithmetic mean, standard deviation and coefficient of variation (CV) were calculated for each trait using the standard formula given in Chandel (1997).

RESULTS

Diversity analysis revealed by chemical parameters: All the results on the chemical parameters studied, with minimum, maximum, mean and CV values, are presented in table 1. In dried fruits, vitamin C was present in non-detectable quantities while minerals like manganese and cobalt were not detected in any sample. The main constituent of the fruits was total carbohydrate with mean value of 73.48±2.43%. The Neutral Detergent Fiber (NDF) contributed most to the total carbohydrate with mean value of 30.48±4.58%. The starch content (15.28±3.15%) was higher than crude fibre (10.94±2.42%). In fruit samples, the structural components like cellulose, hemicellulose and lignin were measured by NDF which contributed in decreasing to NDF with the mean value of 11.45±2.10%, 8.91±1.70% and 7.62±1.04%, respectively. As per coefficient of variation, NDF content was the most variable trait (CV=9.95) followed by starch (CV=6.85) and soluble carbohydrate (CV=6.15). The least variable trait was ash content with the CV value of 0.80. All the minerals except manganese and cobalt were found in detectable quantities. However, calcium (3.24±0.14%) followed by phosphorus (219.05±17.52% [mg/100g]), were found in much higher quantities when compared to other minerals. Ash content showed less CV, however, calcium and phosphorus showed much higher coefficient value; while, the highest CV was obtained for sodium (99.22) followed by phosphorus (38.08).

The Manhattan dissimilarity coefficient values varied between 0.02 (NG44 and NG5) and 0.31 (NG33 and NG18) with an average of 0.092 for chemical parameters. The UPGMA-based dendrogram had clearly put all the individuals into three major groups at an average cut off value (0.092) (Fig. 1a). According to the UPGMA-based clustering, II group had maximum (37) number of samples while, I and III groups accommodated two and seven samples, respectively.

RAPD-based diversity analysis: For the RAPD analysis, 14 polymorphic primers were used which yielded distinct and easily detectable bands. Indistinct bands produced by nonspecific amplification were ignored. Considering all the primers and genotypes, a total of 235 amplicons were obtained, of which 81.27% were observed as polymorphic (Table 2). The number of amplicons produced per primer varied from 9 to 21 with a mean of 16.78 bands per primer. The size of scored bands ranged from 150 to 3 215bp. Maximum percent polymorphism was obtained using primer OPF9 (100%) followed by OPF11 and OPH21 (90%). The discriminating power for RAPD primers ranged from 0.69 (OPG8) to 0.97 (OPF9) (Table 2). The two methods, % polymorphism and discriminating power, were proportionate with only 0.012, correlation coefficient. Jaccard's similarity coefficients among the all pair-wise combinations of genotypes ranged from 0.34 to 0.86, with a mean genetic similarity of 0.50. Maximum similarity (0.86) was found between NG33 and NG48, while the minimum (0.34) was found between NG19 and NG10 (Fig. 1b). Dendrogram based on UPGMA analysis separated all the genotypes into six groups at an average cut-off value of 0.50. Most of the samples were clustered into group one (25 samples), while group five and six contained only a single sample each.

DISCUSSION

With the ever increasing population pressure and fast depletion of natural resources,

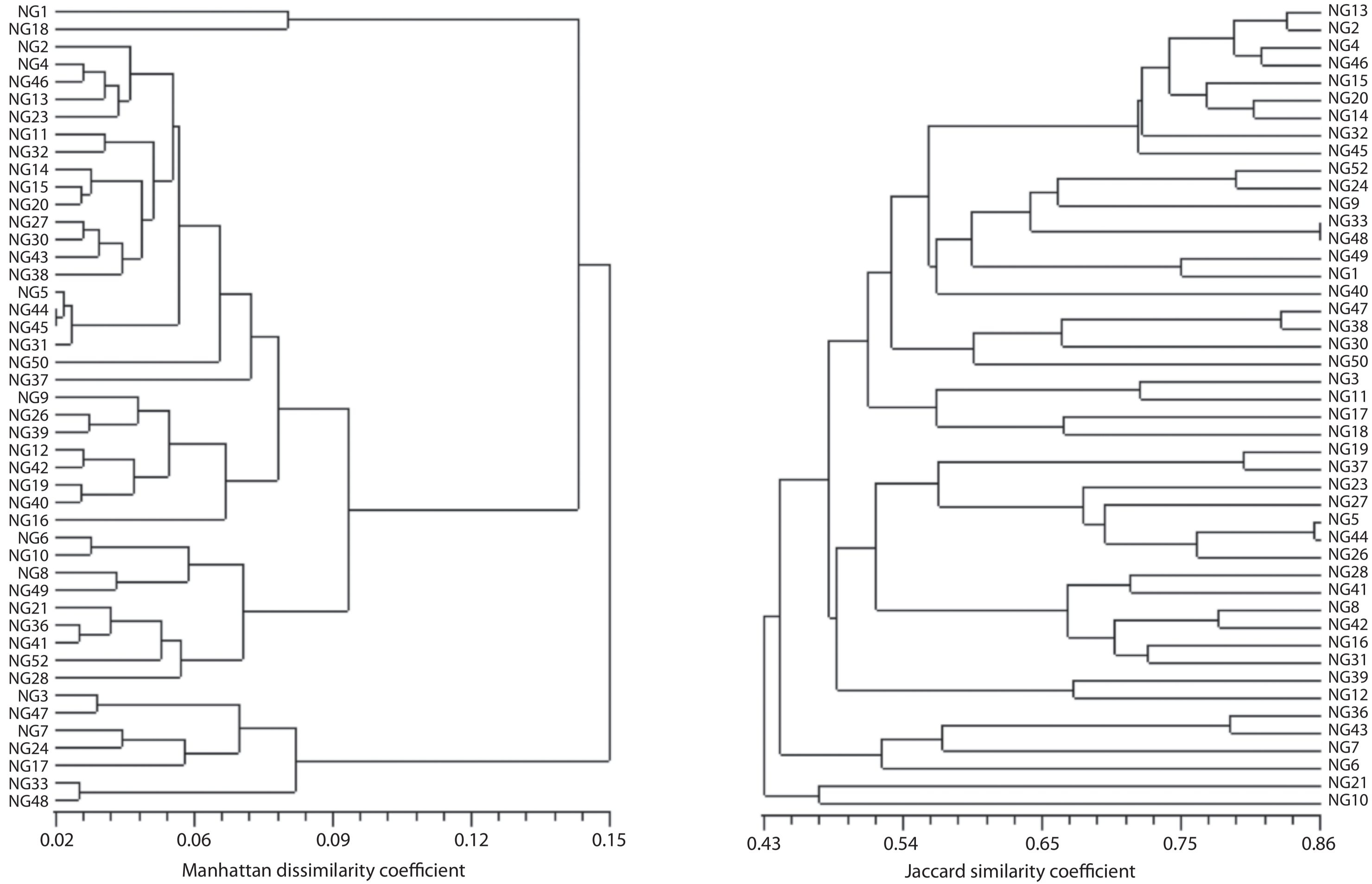

Fig. 1. UPGMA cluster analysis with **A)** Manhattan dissimilarity coefficient of chemical parameters and **B)** Jaccard's similarity coefficient of RAPD based markers.

it has become necessary to evaluate the possibilities of exploiting new plant resources in order to meet the growing needs of the human society, which incidentally has depended only on a small fraction (less than 30 crops) of plant wealth comprising (Anonymous 1975). Many of the under-utilised plant species have great potential for exploitation in view of the value of their economic products for use as food, fodder, medicine, energy and industrial purposes. There is a number of indigenous potential plant species which support life in more extreme environmental situations as species of emergency utility. At the International Workshop on Maintenance and Evaluation of Life Support Species in Asia and the Pacific Region, held at the National Bureau of Plant Genetic Resources, New Delhi in April 1987, a number of species were identified as priority species for further research in view of their economic potential and *C. decidua* was one among them (Paroda *et al.* 1988).

In the present study, an effort was made to analyse the variability of *C. decidua* species from the Thar Desert of Western Rajasthan (India), by analyzing its chemical and genetic diversity. Nutritionally, all the samples were found rich in nutritional compounds indicating high nutritional value of ker fruits under the typical arid conditions of the Desert. Moreover, the good minerals status also supports the survival of plant in its harsher habitat (Rakić *et al.* 2009). A great variability among chemical parameters provides an ample opportunity for their selection in desirable direction. The results of chemical parameters can be used for drug and medicinal values. The low moisture content (63.7-75%) of *C. decidua* plants indicates adaptability and hardiness of *C. decidua* in adverse conditions of the desert with scanty and erratic rainfall. Rosenthal *et al.* (2005) and Vyas *et al*. (2009) also reported low moisture content in the wild stands of desert plants. Lignin and compatible solutes such as proline and glycine-betaine played an important role under abiotic stress in many higher plants (Kumar *et al.* 2011) but higher amount of lignin may hamper efforts to select a genotype with lignin free fruits - an important trait to fetch better market price.

RAPD markers, along with appropriate statistical procedures, are suitable for genetic variation analyses. In the present study, the random RAPD markers were used in combination with nutritional and chemical contents to detect genetic variation of *C. decidua.* All the 14 RAPD primers produced a very good range of discriminatory power which correlates with the assumption that all the primers generated distinct profiles for all the plant samples. The average dissimilarity observed through chemical parameters was lower than the RAPD (50%), suggesting the RAPD as a better technique for genetic diversity estimation. In diversity analysis, multi-fragments nature of RAPD primers also offers advantages over morpho-physiological and chemical characteristics where limited numbers and more similar patterns of their expression restrict high diversity especially among perennial desert plants. Moreover, RAPD amplifies sites from unexpressed as well expressed genome while only coding genome is considered in chemical parameters (Chandra & Dubey 2010). So, the chemical-based diversity reflects expressed genetic composition obviously limiting the range of diversification beyond functional integrity.

Most of the chemical-based variability was supposed to be exhibited by highly variable chemical constituents such as phosphorus, sodium, magnesium and NDF. Structural chemical constituents such as hemicellulose, cellulose and lignin exhibited low values for coefficient of variation and contributed less to chemical-based variability (Kumar *et al.* 2011). A narrowest range of dissimilarity coefficients and greater similarity among wild samples of *C. decidua* was observed on the basis of chemical parameters, whereas, RAPD marker polymorphisms showed a large variability among samples. The chemical constituents of fruits are less affected by the invariable climatic conditions due to the hardy and perennial nature of *C. decidua* moreover, the small area covered under the study for the diversity analysis, might be the reason for very low and narrow ranged dissimilarity coefficients. Another cause was probably that the RAPD revealed the diversity of the entire genome to a greater extent (Kumar & Sharma 2011).

The present study revealed a very low correlation (0.01) between chemical parameters and RAPD-based matrices indicating that the two methods were different and highly variable. Moreover, such a weak correlation could be explained by the different properties of molecular and chemical parameters (Navarro *et al*. 2005). Molecular markers are usually considered selectively neutral and thus do not necessarily reflect the diversity in functional characters (Li *et al.* 2008). Further, such a low correlation suggests differences in the degree of genomic coverage between RAPD and chemical parameters. However, with a low correlation, few samples shared same group in both dendrograms e.g. NG-5/NG44; NG33/NG48; NG13/NG2/NG4/NG46; NG14/NG15/NG20. Low correlation suggests that RAPD-based data are not useful for estimating the chemical characteristics of *C. decidua* samples. Krofta *et al.* (1998) and Patzak *et al.* (2010) also found differences between molecular and chemical data of wild hops (*Humulus lupulus*). Therefore, in order to describe the population diversity for tree and perennial species, it can be strongly recommended to use both morphological and molecular assays as complementary methods.

The chemical and RAPD-based diversity in the present study was found comparable with a previous diversity study for *Calligonum polygonoides* (Vyas *et al.* 2012 in press) and *C. decidua* (Vyas *et al.* 2009). Moreover, the study site of the present investigation and in the study of Vyas *et al.* (2009) consisted of isolated patches of wild populations of *C. decidua* and exhibited nearly the same level of chemical- and RAPD-based diversity. The co-similarity among both studies indicated that, genetically, samples were more diverse but chemical parameters were less influenced by the extremities of Thar Desert.

Chemical analysis revealed that the *C. decidua* samples were rich in nutrition and should be conserved as it is an important food source for mankind and animals in the Thar Desert. Such type of study at a broad level would enable selection of highly nutritive caper plants to fight against malnutrition. It is widely consumed by human populations so highly nutritive caper plants could be used as dietary supplements for those people that cannot afford regular purchase of fresh fruits and vegetables. Moreover, such nutritional rich and diverse *C. decidua* samples can be exploited through tissue culture for reforestation activities in the desert (Tyagi *et al.* 2010) and might be added to fortify nutritionally deficiencies in normal foods of desert areas.

ACKNOWLEDGMENT

Authors are thankful to Department of Biotechnology, Government of India for funding the project “Characterization of underutilized species of the Thar Desert”.

RESUMEN

El desierto de Thar, un lugar muy inhóspito, alberga sólo a las especies de plantas capaces de resistir a condiciones de sequía extrema, a las precipitaciones impredecibles, y a las plantas que pueden crecer en la humedad limitada de los suelos arenosos. *Capparis decidua* se encuentra entre una las pocas plantas capaces de crecer bien en estas condiciones. Esta especie es altamente explotada y se ha tomado de forma natural, así los habitantes locales las han usado para varios propósitos, como alimento, madera y combustible, aunque sin ningún programa de manejo o esfuerzo por conservación. El presente estudio se llevó a cabo en esta zona árida del oeste de Rajastán (India) con el objetivo de obtener información molecular preliminar sobre este grupo de plantas. Se evaluó la diversidad entre 46 muestras de *C. decidua* usando parámetros químicos y marcadores de ADN polimórfico amplificado al azar (RAPD por sus siglas en inglés). Catorce parámetros químicos y ocho minerales (22 variables en total) de los frutos de esta especie fueron estimados. Un total de 14 cebadores para RAPD produjeron 235 posiciones de bandas, de las cuales 81.27% fueron polimórficas. El coeficiente de similitud de Jaccard para los cebadores del RAPD varió entre 0.34 y 0.86 con un promedio de similitud genética de 0.50. De acuerdo con el coeficiente de variación observado, se encontró que el contenido de NDF fue el rasgo más variable, seguido por el almidón y los carbohidratos solubles. Los valores del coeficiente de disimilitud de Manhattan para los parámetros

químicos osciló entre 0.02-0.31 con un promedio de 0.092. El presente estudio reveló una correlación muy baja (0.01) entre los parámetros químicos y las matrices basadas en RAPD. La baja correlación entre las matrices químicas y la basada en RAPD indicó que los dos métodos fueron diferentes y altamente variables. El estudio de la diversidad basada en su química ayudará en la selección de muestras nutricionalmente ricas para fines medicinales, mientras que la diversidad genética ayudará a enfrentar los desafíos naturales y encontrar formas sostenibles para promover la conservación de esta plana para uso futuro.

Palabras clave: *Capparis decidua,* diversidad química, parámetros nutricionales, desierto, RAPD.

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