

RESEARCH ARTICLE

Genetic Diversity Assessment of *Cordia gharaf* (Forsk.) Germplasm for Morpho-Physiological and Fodder Traits in Hot-arid Kachchh Region of India

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Abstract

About 81% of India's arid areas fall in western Rajasthan and Gujarat. In arid regions, *Cordia gharaf* (Forsk.) is one of the important under-utilized multipurpose shrubs that is well adapted to drought, salt, dry winds, and extreme temperatures. This investigation aimed to unveil potential genotypes of species for fruit and fodder use. Sixteen accessions of *C. gharaf* were collected from the hot-arid Kachchh region of Gujarat, India. Collected germplasm was characterized for 14 morpho-physiological and 13 fodder quality traits during the year 2017 – 19, indicating wide variation. Morpho-physiological traits viz. plant height ranged from (155–244.67 cm), canopy spread (140–254 cm²), number of branches per plant (6.67–11.67) leaf area (5.88–11.3 cm²), relative water content (62.79–96.0%), relative stress injury (8.6–17.46%) and canopy temperature depression (2.17–5.83°C). Fodder quality traits like above-ground biomass (AGB) varied from (2.22–19.63 kg/ plant), calcium (0.56–3.70%), crude protein (9.07–17.31%), neutral detergent fiber (NDF, 35.9–68.9%) and acid detergent fiber (ADF, 35.62–58.7%). A high coefficient of variation was recorded for phosphorus (78.85%), AGB/plant (57.79%), silica (36.68%), and calcium (29.55%). The genotypes CG-11, CG-12, CG-13, and CG-15 were reported promising for morphological and above-ground biomass traits, whereas genotypes CG-5, CG-6, and CG-8 reported promising for fodder quality traits.

Keywords: *Cordia gharaf*, Characterization, Genetic diversity, Germplasm, Kachchh.

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Introduction

The hot-arid region of India covers around 31.7 million ha, mainly spread in the states of Rajasthan, Gujarat, Punjab and Haryana, Maharashtra, Karnataka, and Andhra Pradesh. About 81% of this area falls in India's western Rajasthan and Gujarat states (Sharma and Tewari, 2005). These regions are characterized by low and erratic rainfall with a high coefficient of variation and harsh climatic conditions. This hot-arid region is endowed with rich plant diversity and excellent adaptations to survive harsh climatic conditions. Many of these plants are a boon for this region, and the genus *Cordia* has an important role among them. The *Cordia* belongs to the Boraginaceae family and is a large genus comprised of about 300 species of shrubs; small to medium-sized trees have widely been grown in dry and hot regions of the world (Bouby *et al.*, 2011). Around 21 species of this genus have been reported in India (BSI, 2022). Out of these, four species, namely, *Cordia gharaf* (Forsk.), *C. myxa* L., *C. macleodii* (Griff.) Hook. f. et Thomson and *C. sebestena* L. were reported in hot-arid regions of India (Bhandari, 1990). Among them, *C. gharaf* (2n=72), (syn. *C. sinensis* Lam., *C. rothii* Roem. & Schult.), locally known as "Gondi", is one of the important under-utilized multipurpose shrub or small sized tree of this region. Worldwide, it is distributed in drier areas of tropical Africa, Saudi Arabia, Abyssinia, Ceylon, and India (Bhandari, 1990).

In India, it is reported in the states of Rajasthan, Gujarat, Andhra Pradesh, Goa, Haryana, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Tamil Nadu, Uttarakhand, and Uttar Pradesh (BSI, 2022). In the hot-arid Kachchh region of Gujarat, it most occurred on leveled topography on field boundaries and scrub forests with a soil pH of 8 to 8.5 (Dev *et al.*, 2020). It adapted to drought, salt, dry winds, and extreme temperatures due to morphological and physiological adaptability. It is a multi-stemmed shrub or small tree, growing up to 12 m high. Leaves are opposite or sub-opposite, ovate to obovate, and light green with pale hairs on both surfaces. Flowers are small, born in terminal and axillary cymes, white or cream, and are sweetly scented. Flowering and fruiting occurred from March to June. The fruit is 9 to 12 mm long, ovoid, drupe and bright red or yellow-orange color. One fruit contains 1 to 4 hard and rough seeds (Bhandari, 1990; Warfa, 1990; Orwa *et al.*, 2009). This multipurpose species is valued for its edible berries, fuel wood, and leaf fodder, which is available for a long season and has great medicinal importance. The fruits are eaten and sold in local markets. The children chew the bark to redden their lips as a substitute for *Pan*. The wood is used for fuel and in the manufacture of agricultural implements. The decoction of the bark is used for gargles. It has great potential to withstand abiotic pressure and reduce soil erosion because of its strong tap root system, providing livelihood security against drought and famine in the hot-arid Kachchh region of India. Leaves are mainly used as fodder by browsing animals such as goats, sheep, and camels in hot-arid regions. Its leaves are a good source of crude protein (13.8%) and dry matter (41.2%), neutral detergent fiber (NDF, 56.6%), acid detergent fiber (ADF, 43.3%), and acid detergent lignin (ADL, 18.9%) with high amount of minerals like calcium, zinc, potassium, phosphorus, and iron. Its fruits are also a rich source of energy (18.9 kcal/100 g dry wt.), crude protein (12.6%) and dietary fiber (11.6%) (Kuria *et al.*, 2005).

C. gharaf is an important fodder shrub for small ruminants, and hot-arid regions have a wide genetic diversity for the traits. As a multipurpose and under-utilized fodder and fruit shrub species in the hot-arid region of India, it is very important to collect available genetic diversity from natural habitats and characterize them for different traits for genetic improvement and further exploitation. But more scientific information is needed on germplasm collection, characterization and utilization of this species. Further, on-farm conservation and studying the genetic diversity of this under-explored shrub is very important for the identification of superior genotypes for its exploitation and utilization in alternate land use systems. Thus, the present study was carried out to collect available germplasm from the hot-arid Kachchh region of India and characterize them for further genetic improvement and utilization.

Materials and Methods

Field survey and Germplasm Collection

Field surveys were carried out in five talukas, namely Bhuj, Mandvi, Nakhatarana, Bhachau, and Rapar of arid Kachchh, Gujarat, during summer (April - May) and monsoon (July - September) seasons of two consecutive years, 2015 and 2016 for collection of germplasm of *C. gharaf*. The passport information was also collected during germplasm collection.

Evaluation of Morpho-physiological and Fodder Quality Traits

Collected seeds were sown in polythene bags, and seedlings were raised in the nursery. A total of sixteen survived germplasm lines were transplanted in the experimental field at 6×6 m spacing during August - September 2016 using RBD with five replications at the age of eight months at ICAR-Central Arid Zone Research Institute, Regional Center, Kukma-Bhuj, Gujarat for evaluation. The field evaluation, flowering, fruiting and seed photograph are presented in Fig. 1. Growth pattern traits like plant height, canopy spread, number of leaves/plant, and stem thickness were recorded at regular one-year intervals up to three years during the years 2017, 2018 & 2019 after the monsoon season. Observations were recorded for 14 morpho-physiological and 13 fodder quality traits after three years of transplanting in the field after the monsoon season (Mahajan *et al.*, 2002). The leaf area of individual genotypes was measured by taking third newer leaves from the top of five different branches with the help of a leaf area meter (CI-203 laser area meter, CID, Inc. USA).

Relative water content (RWC)

The RWC expresses the water content (%) at a given time as related to the water content at full turgor. RWC was estimated as follows (González and González-Vilar, 2001).

$$RWC (\%) = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

Similarly, leaves' dry weight and moisture content were calculated by weighing the fresh leaf weight and oven-dry weight (samples oven-dried for 72 hours at a constant temperature of 72°C) as per the procedure advocated by van de Sande-Bakhuyzen (1928).

Canopy Temperature Depression (CTD)

CTD is calculated as the difference between air temperature and canopy temperature using an infrared thermometer (Balota *et al.*, 2008).

$$CTD = T_{\text{air}} - T_{\text{canopy}}$$

Relative Stress Injury (RSI)

RSI is estimated as per the procedure of Dionisio and Tobita (1998). The leaf sample (0.1 g) from three randomly selected plants was cut into uniform pieces. The samples were set in 10 ml of double distilled water in two sets. One set was kept at 40°C for 30 minutes, and its conductivity (ECa) was measured. The subsequent set was held in a boiling water bath (100°C) for 10 minutes and its conductivity (ECb) was noted. RSI was calculated as follows;

$$\text{RSI (\%)} = \frac{\text{ECa}}{\text{ECa} + \text{ECb}} \times 100$$

Fodder Nutrient Analysis

Total ash was determined according to the AOAC (1990), nitrogen was estimated using the Kjeldahl method (AOAC, 2019) and crude protein was determined by increasing the nitrogen content with a factor of 6.25. The NDF and ADF were estimated as suggested by Van Soest *et al.* (1991). All the samples were analyzed thrice for nutrient analysis in *C. gharaf* leaves.

Statistical Analysis

The experiment was planned with a complete randomized block design in five replications. The mean values were used for the statistical analysis of recorded trial data for 14 morpho-physiological and 13 fodder quality traits tested at the hot-arid region of Kachchh. The plant growth and fodder quality characteristics were taken in five and three repetitions, respectively. The descriptive statistics of studied traits were derived using IBM SPSS statistics software version 20.0 (IBM SPSS, 2011). Pearson's correlation coefficient, cluster, and biplot analysis used R software version 3.6.1 (R Core Team, 2019). The "PAST4" program was used to perform principal component analysis (Hammer *et al.*, 2001).

Results and Discussion

Field survey, collection and distribution pattern

During the field survey, 20 distinct germplasm in the form of ripened fruits were collected from different habitats. Its distribution was found in various natural habitats like wild areas, field boundaries, backyards, roadsides, and near human settlements in Bhuj, Rapar, and Mandvi mandals of the Kachchh district. The passport information of collection sites was also collected and is presented in Table 1.

Growth pattern of *C. gharaf* from the year 2017 to 2019

The growth pattern of *C. gharaf* germplasm showed good plant growth concerning plant height (cm), canopy spread (cm²), number of leaves per plant and stem thickness (mm) during the years 2017 to 2019 in the field (Table 2). The plant height increased up to 206.3 cm after the third year

of planting in 2019 and varied from 41 to 120 cm, 121.7 to 212 cm, and 155 to 244.7 cm during 2017, 2018, and 2019, respectively. The canopy spread ranged from 27.5 – 73.0 cm², 118 to 223 cm², and 140 to 254 cm², with mean values of 48.8 cm², 177.5 cm² and 203.3 cm² during 2017, 2018 and 2019, respectively. The number of leaves per plant increased considerably up to the third year in 2019, reaching a value of 4201 leaves per plant. The stem thickness also substantially increased to 32.8 mm after the third year of planting in 2019. The abundant plant growth of this species in adverse climatic conditions of hot-arid regions makes it suitable for introduction in the rangeland improvement programs.

Genetic variability for morpho-physiological traits

Descriptive statistics carried out for 14 morpho-physiological traits of sixteen *C. gharaf* accessions showed a wide range of variability (Table 3 and Supplementary Table 1). The plant height and canopy spread ranged from 155 to 244.67 cm and 140 to 254 cm², respectively, with a mean value of 206.33 cm and 203.3 cm², respectively. The number of branches and leaves per plant range from 6.67 to 11.67 and 2430 to 6047.33, respectively, with a mean value of 8.81 and 4201.33. Similarly, leaf length and width ranged from 5.21 to 6.63 cm and 7.57 to 9.57 cm, respectively, with a mean of 5.78 cm and 8.75 cm, respectively. The trait stem thickness was recorded between 21.59 and 46.0 mm, with a mean value of 32.8 mm. Petiole length was measured between 1.97 and 2.93 cm with a mean of 2.5 cm, whereas petiole width was recorded in the 3.22 and 4.18 mm range with a mean of 3.67 mm. The important trait contributing mainly to photosynthesis and biomass production, i.e., leaf area, varied between 5.88 and 11.30 cm² with an average value of 9.35 cm².

A considerable level of variation was found among the physiological traits that were evaluated in the third year. These characteristics showed how resilient and adaptable a genotype is under stress conditions. The RWC, CTD, and RSI values range from 62.79 to 96%, 2.17 to 5.83°C, and 8.6 to 17.46%, with a mean value of 71.18%, 3.72°C, and 13.33%, respectively.

Among the tested morpho-physiological traits, the highest coefficient of variation (CV) was recorded for CTD (23.99%) followed by the number of leaves per plant (23.28%). The lowest CV was observed for leaf width (6.01%), followed by leaf length (6.95%) and petiole width (7.53%).

Among the studied genotypes, the genotype CG-13 collected from the roadside of Mandvi showed maximum plant height (244.67 cm) followed by CG-14 (243.33 cm) and minimum CG-02 (155.0 cm). Similarly, the maximum canopy spread was noted for genotype CG-11 (254.0 cm²), followed by CG-13 (249.33 cm²) and minimum in CG-02 (140.0 cm²) in the third year of evaluation. The genotype with the maximum leaf area was CG-12 (11.30 cm²), followed by CG-16 (10.80 cm²), and the minimum was CG-01 (5.88 cm²). The highest RWC was recorded for CG-16 (96.0%), followed by

Table 1: Passport information of 16 *C. gharaf* (CG) genotypes collected from Kachchh region of Gujarat

Genotypes	Mandal	District	Latitude	Longitude	Habitat
CG-01	Bhuj	Kachchh	23.12330	69.78845	Wild*
CG-02	Bhuj	Kachchh	23.23361	69.73668	Field boundary**
CG-03	Bhuj	Kachchh	23.23561	69.73169	Field boundary
CG-04	Rapar	Kachchh	23.54710	70.73110	Backyard
CG-05	Rapar	Kachchh	23.56288	70.70563	Road side
CG-06	Bhuj	Kachchh	23.18147	69.66737	Wild
CG-07	Bhuj	Kachchh	23.18124	69.66693	Wild
CG-08	Bhuj	Kachchh	23.18139	69.66775	Wild
CG-09	Bhuj	Kachchh	23.21644	69.72455	Field boundary
CG-10	Bhuj	Kachchh	23.21576	69.72282	Field boundary
CG-11	Bhuj	Kachchh	23.19508	69.85644	Forest nursery
CG-12	Mandvi	Kachchh	23.08289	69.52191	Road side
CG-13	Mandvi	Kachchh	22.89927	69.39358	Road side
CG-14	Mandvi	Kachchh	22.89152	69.40769	Road side
CG-15	Mandvi	Kachchh	22.84624	69.37469	Near human settlement
CG-16	Mandvi	Kachchh	22.85733	69.29186	Wild

*'Wild' is used about an area of land in its natural state, not changed by people.

**'Field boundaries' are the geometric borders of agricultural fields. A field, in this case, is an area of land, enclosed or otherwise, used for agricultural purposes such as cultivating crops.

Table 2: Morphological growth pattern of *C. gharaf* during year 2017, 2018 & 2019

Traits	Years	Range		Mean \pm SEM	SD	CV (%)
		Min.	Max.			
Plant height (cm)	2017	41.0	120.0	83.3 \pm 6.1	24.4	29.2
	2018	121.7	212.0	162.6 \pm 6.9	27.4	16.9
	2019	155.0	244.7	206.3 \pm 7.3	29.4	14.2
Canopy spread (cm ²)	2017	27.5	73.0	48.8 \pm 4.2	16.8	34.4
	2018	118.0	223.0	177.5 \pm 7.8	31.0	17.5
	2019	140.0	254.0	203.3 \pm 8.7	34.9	17.2
No. of leaves/plant	2017	91.7	620.0	251.6 \pm 41.0	164.0	65.2
	2018	322.3	2991.3	1271.6 \pm 211.7	846.9	66.6
	2019	2430.0	6047.3	4201.3 \pm 244.6	978.2	23.3
Stem thickness (mm)	2017	6.4	14.4	10.3 \pm 0.6	2.3	22.6
	2018	17.5	38.7	26.4 \pm 1.5	6.2	23.3
	2019	21.6	46.0	32.8 \pm 1.8	7.1	21.5

CG-13 (90.0%), and the minimum was for CG-14 and CG-12 (62.79% each). Likewise, the highest CTD was recorded for genotype CG-03 (5.83°C), followed by CG-02 (4.77°C). The genotype CG-12 showed the highest (17.46%) RSI, followed by CG-01 (16.72%) and minimum in genotype CG-07 (8.6%). By looking at the growth performance of *C. gharaf* in the adverse conditions of hot-arid Kachchh of Gujarat, it might be used in silvipasture systems or rangeland improvement programs for sustainable development in hot-arid regions of India.

Meghwal *et al.* (2014) reported the presence of wide variability in important fruit characters viz., bunch weight, number of fruits per bunch, fruit weight and pulp: stone ratio of 14 *C. myxa* L. germplasm collected from Rajasthan and Gujarat. Earlier studies also revealed the wide genetic diversity of *C. myxa* in natural populations for its morphological traits (Pareek and Sharma, 1993; Saini *et al.*, 2002; Samadia, 2005; Malik *et al.*, 2010; Sivalingam *et al.*, 2012). The morphological and molecular (RAPD marker) diversity study in 22 *Cordia* accessions of the Indian hot-arid

Table 3: Descriptive statistics of 14 morpho-physiological and 13 fodder quality traits recorded in 16 *C. gharaf* accessions

S No.	Traits	Range		Mean \pm SEM	SD	CV (%)
		Mini.	Maxi.			
Morpho-physiological traits						
1.	Plant height (cm)	155.00	244.67	206.33 \pm 7.34	29.36	14.23
2.	Canopy spread (cm ²)	140.00	254.00	203.3 \pm 8.7	34.9	17.2
3.	No. of branches/plant	6.67	11.67	8.81 \pm 0.37	1.50	16.97
4.	No. of leaves/plant	2430.00	6047.33	4201.33 \pm 244.55	978.21	23.28
5.	Stem thickness (mm)	21.59	46.0	32.8 \pm 1.8	7.1	21.5
6.	Leaf length (cm)	5.21	6.63	5.78 \pm 0.10	0.40	6.95
7.	Leaf width (cm)	7.57	9.57	8.75 \pm 0.13	0.53	6.01
8.	Petiole length (cm)	1.97	2.93	2.50 \pm 0.07	0.27	10.94
9.	Petiole width (mm)	3.22	4.18	3.67 \pm 0.07	0.28	7.53
10.	Fresh leaf weight (10 leaves)	1.85	3.15	2.44 \pm 0.09	0.34	14.10
11.	Leaf area (cm ²)	5.88	11.30	9.35 \pm 0.34	1.37	14.63
12.	RWC (%)	62.79	96.00	71.18 \pm 2.39	9.55	13.42
13.	CTD (°C)	2.17	5.83	3.72 \pm 0.22	0.89	23.99
14.	RSI (%)	8.60	17.46	13.33 \pm 0.70	2.79	20.92
Fodder quality traits after three years of transplanting						
1.	AGB/plant (kg)	2.22	19.63	8.08 \pm 1.17	4.67	57.79
2.	Moisture content (%)	55.41	72.51	65.93 \pm 1.17	4.69	7.11
3.	Dry weight (%)	27.49	44.59	34.07 \pm 1.17	4.69	13.77
4.	Total ash (%)	6.65	18.20	13.55 \pm 0.80	3.18	23.50
5.	Organic matter (%)	81.65	93.10	86.32 \pm 0.78	3.11	3.60
6.	Carbon (%)	41.10	46.70	43.55 \pm 0.39	1.57	3.62
7.	Silica (%)	0.65	3.35	1.78 \pm 0.16	0.65	36.68
8.	Calcium (%)	0.56	3.70	2.73 \pm 0.20	0.81	29.55
9.	Phosphorus (%)	0.07	0.68	0.18 \pm 0.03	0.14	78.85
10.	N ₂ (%)	1.45	2.77	1.72 \pm 0.09	0.36	21.04
11.	CP (%)	9.07	17.31	10.75 \pm 0.57	2.26	21.04
12.	NDF (%)	35.90	68.90	50.51 \pm 2.05	8.20	16.24
13.	ADF (%)	35.62	58.70	48.87 \pm 1.60	6.38	13.06

RWC-Relative water content, CTD-Canopy temperature depression, RSI-Relative stress injury, AGB-Above ground biomass, CP-Crude protein, NDF-Neutral detergent fiber, ADF-Acid detergent fiber

region revealed considerable genetic diversity in studied accessions (Sivalingam *et al.*, 2012). Kumar *et al.* (2017) studied the morphometric variability in *C. gharaf* collection, especially for seed, fruit and seedling traits from the Jaisalmer district. They found that the Badabagh collection (CAZJCG-1) performed better concerning seed germination and seedling growth parameters. The study of other under-utilized hot-arid shrub species like *Grewia tenax* was also carried out by Patidar *et al.* (2022) using morphological and phytochemical traits under hot-arid condition of Jaisalmer and observed the highest CV for number of branches per plant (31.82%) followed by plant height (23.25%). Similarly, Dev *et al.* (2017) also studied the diversity of *G. tenax* using morphological and fruit traits after one year of planting

Table 4: Distribution of 16 *C. gharaf* (CG) accessions into different clusters

Clusters	Number of genotypes	Genotypes
I	2	CG-01, CG-02
II	7	CG-03, CG-06, CG-09, CG-10, CG-11, CG-12, CG-15
III	3	CG-04, CG-05, CG-08
IV	4	CG-07, CG-13, CG-14, CG-16

in hot-arid climatic conditions in Kachchh, Gujarat. They reported diverse plant heights from 20.8 to 78.5 cm among the different genotypes.



Fig. 1: Field evaluation of *Cordia gharaf* at ICAR-Central Arid Zone Research Institute, Regional Center, Kukma-Bhuj, Gujarat (a), Flowering twig (b), Fruiting branches (c), Seeds of *C. gharaf* (d)

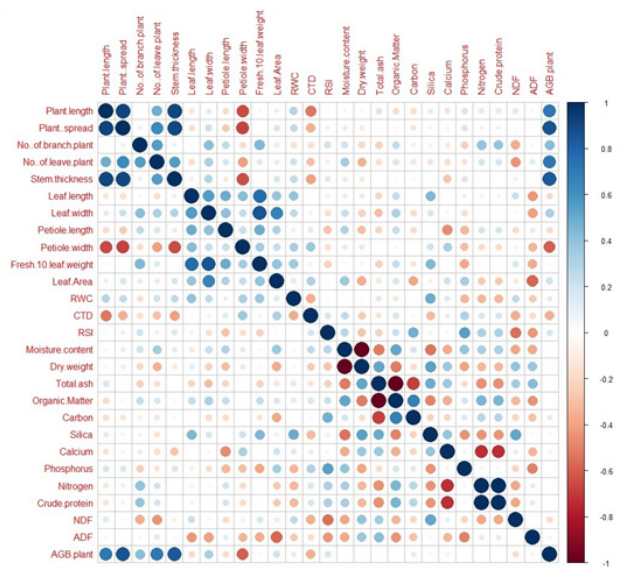


Fig. 2: Pearson's correlation coefficient plot of 16 *Cordia gharaf* accessions for 27 morpho-physiological and fodder quality traits

Genetic variability for fodder quality traits

Descriptive statistics for 13 fodder quality traits in sixteen *C. gharaf* accessions showed a wide range of variability among them (Table 3 and Supplementary Table 2). Above-ground biomass (AGB) per plant ranged from 2.22 to 19.63 kg/plant with a mean value of 8.08 kg/plant. Similarly, leaf moisture content varied between 55.41 to 72.51% and dry matter in the leaves ranged from 27.49 to 44.59%. The maximum organic matter and carbon content were estimated in genotype CG-08 (93.1 and 46.7%) and minimum in CG-01 (81.65) and CG-02 (41.1%), respectively. The silica content in the leaves ranges between 0.65 and 3.35%. In comparison, calcium content in leaves ranged from 0.56 to 3.7%. Nitrogen and crude protein are two important parameters of quality fodder of any plant species. In this study, these two contents varied from 1.45 to 2.77% and 9.07 to 17.31%, respectively,

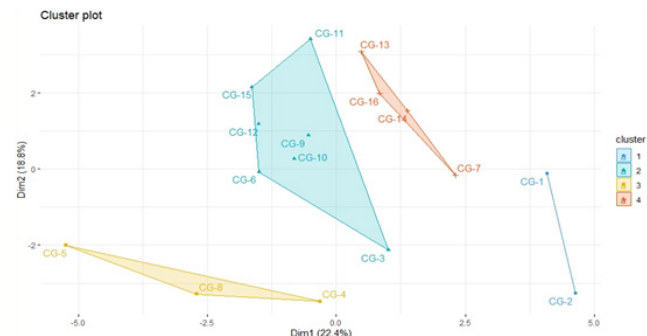


Fig. 3: Cluster plot of 16 *Cordia gharaf* accessions using 27 morpho-physiological and fodder quality traits

and found minimum in CG-11 and CG-01 (1.45 and 9.07%) genotypes and maximum in CG-05 (2.77 and 17.31%) followed by CG-15 (2.23 and 13.93%), respectively. The NDF and ADF content ranges between 35.9 to 68.9% and 35.62 to 58.7%, respectively.

Among the fodder quality traits, the highest CV was recorded for phosphorous content (78.85%), followed by AGB per plant (57.79%) and silica (36.68%). At the same time, the lowest CV was observed for organic matter (3.60%) followed by carbon content (3.62%) and moisture content (7.11%). Kuria et al. (2005) also studied the nutritive value of different range forage species. They reported that the leaves of *C. sinensis* are a good source of crude protein (13.8%), dry matter (41.2%), NDF (56.6%), ADF (43.3%) and ADL (18.9%) with high amounts of minerals like calcium, zinc, potassium, phosphorus, and iron. Jain and Patel (2016) estimates of descriptive statistics for nine quantitative parameters revealed wide variation among sorghum genotypes. Similarly, Dev et al. (2023) found significant variety in *Grewia tenax* germplasm for fodder and related quality parameters. Above-ground biomass (AGB) ranged from 0.4 to 2.9 kg/plant, with significant variance in crude protein (2.2–15.2%), NDF (37.264.2%), and ADF (12.0–47.6%) levels. The fodder quality value can indicate its suitability as a feed source for browsing animals and in silvipasture systems within India's hot-arid regions.

Pearson's correlation of morpho-physiological and fodder quality traits

Pearson's correlation coefficients were estimated among 27 morpho-physiological and fodder quality traits in sixteen *C. gharaf* genotypes (Fig. 2). Among the various characteristics studied, plant height was positively correlated with canopy spread, stem thickness, above-ground biomass per plant, and a number of leaves per plant. Likewise, canopy spread was also positively correlated with stem thickness, above-ground biomass per plant, and number of leaves per plant. The fodder-yielding traits like above-ground biomass were positively associated with morphological traits like plant height, canopy spread, number of branches per plant, number of leaves per plant, and stem thickness; all these

traits contribute to higher plant biomass. Within fodder quality traits, a strong positive correlation was observed between nitrogen content and crude protein. Conversely, significant negative correlations were identified between dry weight and moisture content, as well as between total ash and organic matter (Fig. 2). The correlation studies conducted have revealed a positive correlation between fodder-yielding traits, such as above-ground biomass, and the associated morphological traits. These findings suggest that selecting and emphasizing these specific traits could be highly beneficial in developing cultivars with high fodder yield potential. Guleria *et al.* (2020) tested *Grewia optiva* clones for fodder yield and other features and discovered a favorable relationship between collar diameter, fresh fodder weight, and fuel wood weight. Sivalingam *et al.* (2016) found a positive association between leaf, fruit, stone characteristics, and pulp: stone ratio but a negative or negligible correlation with flower count per cyme in *C. myxa* germplasm. According to the correlation study by Jain and Patel (2016), the fodder yield of sorghum was positively connected with the number of leaves per plant, leaf breadth, leaf length, plant height, and days to maturity. The good correlation among these parameters indicated that they are crucial for the direct selection of high forage-producing genotypes.

Cluster analysis

The cluster plot was generated using 27 morpho-physiological and fodder quality traits of sixteen *C. gharaf* genotypes based on Ward’s approach and grouped all the studied genotypes into four major clusters (Table 4 and Fig. 3). The first cluster consists of only two genotypes and contributed only 12.5% of the total genotypes. On the other hand, the second cluster included seven genotypes with 43.75% of the total genotypes. The clusters third and fourth consisted of 3 and 4 genotypes, respectively. The genotypes included in the same cluster are similar and different to the genotypes of other clusters. Sivalingam *et al.* (2012) discovered that the clustering pattern is mostly determined by leaf size and pulp:stone ratio, rather than the region from where seeds were collected. They may have evolved from various beginnings by diverging from a common ancestor and establishing their own niche. This difference might be attributed to seed-grown plants, cross-pollination, and seed dispersal by birds, animals, and humans from one location to another. Cluster analysis divided the *G. tenax* genotypes into two primary clusters, each with two sub-clusters, confirming the presence of a substantial degree of genetic variability among the tested genotypes (Dev *et al.*, 2023).

Biplot analysis

PCA biplot visually represents the distribution and diversity of both studied traits and the *C. gharaf* germplasm (Fig. 4). The biplot provides insights into the patterns and

relationships between these variables, allowing for a better understanding of their interplay. This also aids in selecting genotypes or groups of genotypes with superior traits, facilitating their selection and utilization for species improvement. In contrast, traits heading in the same direction show a positive correlation, while traits in opposite directions represent a contrast or negative correlation between variables. This analysis provides valuable insights into trait relationships and their impact on species enhancement. The high variance was observed for plant height, plant spread, stem thickness, above-ground biomass (AGB) per plant, number of leaves per plant, moisture content, nitrogen content, crude protein, organic matter, petiole length, dry weight, and total ash. The leaf area, leaf length, leaf width, fresh ten leaves weight, petiole length, phosphorus, and RSI exhibited comparatively lower variance in the observed data. Fig. 4 shows that traits like above-ground biomass, petiole width, total ash and organic carbon are in opposite directions and form an independent group. The traits like AGB per plant, plant spread, plant height, stem thickness and number of leaves per plant were positively correlated and grouped in the same AGB group. Similarly, the total ash content of the genotypes is positively associated with fodder quality traits like silica content, ADF, NDF and dry weight. The Eigenvalue and principal component (PC) number were graphed to generate the scree plot (Fig. 5). It explains the variance associated with each PC. The first four

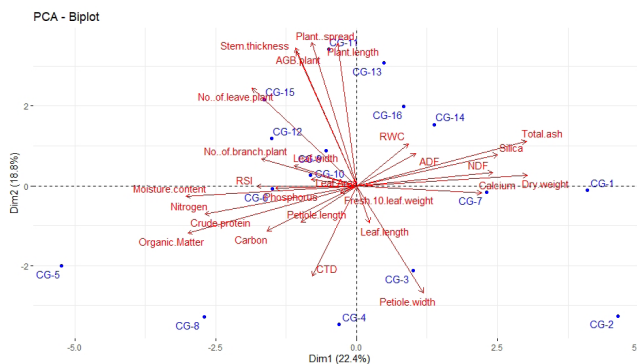


Fig. 4: Biplot

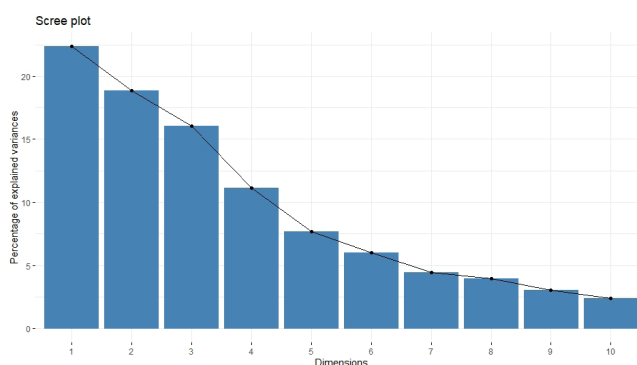


Fig. 5: Scree plot

PCs contributed 68.25% of the total variation revealed in 16 *C. gharaf* germplasm. The initial four principal components (PCs) contain the maximum variation in the data. Therefore, selecting accessions from these PCs would be beneficial for further studies and analyses. The plotting of variables against genotypes and correlation study suggests that the genotypes CG-11, CG-12, CG-13, and CG-15 exhibit favorable characteristics for morphological traits and above-ground biomass per plant. Genotypes CG-05, CG-06, and CG-08 also show good characteristics for fodder quality. These genotypes can be selected as promising candidates for future improvement programs. PCA biplot was used to order the genotypes and to visualize the genetic relationships among accessions. Jain and Patel (2016) discovered that biplot between PCs 1 and 2, distributed genotypes in all four quadrants, indicating high genetic variability in quantitative features in sorghum germplasm. Dev *et al.* (2023) found distinct genotypes (GT-15, GT-1, GT-3, GT-11, GT-13, GT-29, GT-32, GT-33, and GT-36) in *Grewia tenax* through biplot analysis using PC1 and PC2 information.

Conclusion

C. gharaf, a versatile and underexplored/underutilized shrub/small tree species, thrives well in harsh climatic conditions characterized by frequent droughts and crop failures. It is valued for its edible berries, fuel wood and leaf fodder, which is available for a long season and has great medicinal importance. The species' leaves are essential fodder for browsing animals like goats, sheep, and camels in hot-arid regions. Given its importance in challenging environments, it is crucial to characterize the existing variability of heterogeneous germplasm to tap the species' potential. The present study demonstrated a wide range of diversity for morpho-physiological and fodder quality traits in studied germplasm and *ex-situ* on-farm conservation for further improvement for exploitation of its untapped potential value. The promising genotypes identified for morphological, above-ground biomass traits and fodder quality traits can be used in silvipasture systems in hot-arid regions of India.

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Supplementary Table 1: Morpho-physiological traits along with descriptive statistics of 16 *C. gharaf* accessions

Acc.	Plant height (cm)	Canopy spread (cm ²)	No. of branches/plant	No. of leaves/plant	Stem thickness (mm)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Petiole width (mm)	fresh leaf weight (10 leaves)	Leaf Area (cm ²)	RWC (%)	CTD (°C)	RSI (%)
CG-01	202.33	196.8	6.67	2763.33	30.82	5.34	7.57	1.97	3.67	1.85	5.88	76.46	3.00	16.72
CG-02	155.00	140.00	8.00	2430.00	21.59	6.25	8.53	2.60	4.12	2.46	10.05	68.29	4.77	11.55
CG-03	174.67	181.33	7.67	3955.00	25.40	5.49	8.90	2.57	3.94	2.42	9.82	66.07	5.83	10.80
CG-04	168.33	165.33	8.67	3255.00	23.05	5.81	9.03	2.23	4.18	2.67	9.91	75.44	3.90	12.50
CG-05	195.33	186.33	8.67	4206.67	33.09	5.69	8.60	2.70	3.73	2.19	9.01	69.35	4.60	16.70
CG-06	196.00	177.67	11.00	5107.67	33.01	5.64	9.30	2.57	3.76	2.65	9.55	63.64	3.27	14.08
CG-07	202.00	181.33	8.33	3881.67	27.47	5.92	8.40	2.57	3.64	2.42	8.75	71.70	2.17	8.60
CG-08	167.00	166.67	9.00	4156.67	27.15	6.46	8.90	2.93	3.75	2.68	9.22	66.67	4.43	15.14
CG-09	219.33	215.33	9.33	5318.00	29.72	5.21	7.80	2.50	3.32	2.04	7.00	64.58	3.30	12.77
CG-10	205.67	191.33	10.67	4175.00	32.22	5.57	8.77	2.90	3.56	2.47	10.56	69.57	3.57	13.69
CG-11	226.33	254.00	10.00	6047.33	45.99	5.25	8.70	2.23	3.51	2.05	10.16	71.43	3.53	14.72
CG-12	241.33	240.33	7.00	3629.00	39.87	5.66	8.47	2.10	3.32	2.00	11.30	62.79	4.37	17.46
CG-13	244.67	249.33	7.00	5023.33	43.06	5.88	9.10	2.60	3.56	2.53	9.77	90.00	2.80	9.74
CG-14	243.33	228.00	7.67	3492.67	38.37	5.85	9.10	2.60	3.54	2.55	9.36	62.79	3.47	8.90
CG-15	218.00	241.00	11.67	4610.00	37.61	5.88	9.23	2.27	3.22	2.85	8.50	64.10	3.37	14.63
CG-16	242.00	237.33	9.67	5170.00	36.84	6.63	9.57	2.73	3.95	3.15	10.80	96.00	3.20	15.23
Mini.	155.00	140.00	6.67	2430.00	21.59	5.21	7.57	1.97	3.22	1.85	5.88	62.79	2.17	8.60
Maxi.	244.67	254.00	11.67	6047.33	46.02	6.63	9.57	2.93	4.18	3.15	11.30	96.00	5.83	17.46
Range	89.67	114.00	5.00	3617.33	21.47	1.42	2.00	0.97	0.96	1.30	5.42	33.21	3.67	8.86
Mean	206.33	203.32	8.81	4201.33	32.80	5.78	8.75	2.50	3.67	2.44	9.35	71.18	3.72	13.33
SEM	7.34	8.71	0.37	244.55	1.8	0.10	0.13	0.07	0.07	0.09	0.34	2.39	0.22	0.70
SD	29.36	34.90	1.50	978.21	7.1	0.40	0.53	0.27	0.28	0.34	1.37	9.55	0.89	2.79
CV (%)	14.23	17.2	16.97	23.28	21.5	6.95	6.01	10.94	7.53	14.10	14.63	13.42	23.99	20.92

RWC-Relative water content, CTD-Canopy temperature depression, RSI-Relative stress injury

Supplementary Table 2: Fodder quality traits along with descriptive statistics of 16 *C. gharaf* accessions

Acc.	AGB/plant (kg)	Moisture content (%)	Dry weight (%)	Total ash (%)	Organic matter (%)	Carbon (%)	Silica (%)	Calcium (%)	Phosphorus (%)	N ₂ (%)	CP (%)	NDF (%)	ADF (%)
CG-01	4.82	58.93	41.07	18.10	81.65	45.95	2.65	3.34	0.14	1.45	9.07	55.10	53.10
CG-02	2.22	55.41	44.59	18.20	82.15	41.10	2.25	3.66	0.12	1.55	9.69	53.20	51.70
CG-03	3.29	70.16	29.84	16.15	83.60	41.68	1.65	3.18	0.14	1.59	9.92	51.35	52.10
CG-04	2.97	67.91	32.09	8.35	91.45	45.68	1.45	3.70	0.15	1.59	9.95	55.10	35.62
CG-05	6.95	72.51	27.49	9.10	90.65	45.45	0.65	0.56	0.27	2.77	17.31	42.25	48.75
CG-06	8.78	66.44	33.56	14.10	85.75	42.95	1.35	2.68	0.16	1.97	12.29	35.90	50.50
CG-07	4.77	64.70	35.30	15.05	84.95	42.73	1.95	2.31	0.13	1.55	9.68	68.90	52.10
CG-08	4.19	68.43	31.57	6.65	93.10	46.70	1.55	2.50	0.20	1.77	11.07	40.50	45.90
CG-09	6.75	65.92	34.08	14.05	85.75	42.95	1.25	2.53	0.13	2.02	12.61	45.30	58.70
CG-10	8.54	72.28	27.72	14.95	85.10	42.55	1.95	2.42	0.15	1.54	9.60	47.30	48.75
CG-11	19.63	64.02	35.98	13.30	86.45	43.35	1.55	3.56	0.15	1.45	9.07	42.30	51.25
CG-12	9.12	70.58	29.42	13.55	86.35	43.38	1.05	3.42	0.68	1.48	9.26	48.75	37.80
CG-13	11.65	66.53	33.47	14.45	85.40	42.98	1.50	2.22	0.07	1.46	9.11	52.20	53.20
CG-14	10.18	60.56	39.44	13.35	86.35	43.28	2.05	2.07	0.11	1.58	9.90	59.80	54.70
CG-15	14.80	64.39	35.61	12.55	87.30	43.60	2.25	2.32	0.14	2.23	13.93	55.05	48.90
CG-16	10.64	66.06	33.94	14.90	85.10	42.55	3.35	3.28	0.11	1.53	9.53	55.10	38.90
Mini.	2.22	55.41	27.49	6.65	81.65	41.10	0.65	0.56	0.07	1.45	9.07	35.90	35.62
Maxi.	19.63	72.51	44.59	18.20	93.10	46.70	3.35	3.70	0.68	2.77	17.31	68.90	58.70
Range	17.41	17.10	17.10	11.55	11.45	5.60	2.70	3.14	0.60	1.32	8.24	33.00	23.08
Mean	8.08	65.93	34.07	13.55	86.32	43.55	1.78	2.73	0.18	1.72	10.75	50.51	48.87
SEM	1.17	1.17	1.17	0.80	0.78	0.39	0.16	0.20	0.03	0.09	0.57	2.05	1.60
SD	4.67	4.69	4.69	3.18	3.11	1.57	0.65	0.81	0.14	0.36	2.26	8.20	6.38
CV (%)	57.79	7.11	13.77	23.50	3.60	3.62	36.68	29.55	78.85	21.04	21.04	16.24	13.06

AGB-Above ground biomass, CP-Crude protein, NDF-Neutral detergent fiber, ADF-Acid detergent fiber