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Genetic Diversity among Wild *Grewia tenax* Accessions Collected from Kachchh Region of Gujarat, India

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The germplasm of *Grewia tenax* (Phalsa cherry or white cross-berry) as fruits were collected from arid region of Kachchh, Gujarat during the year 2015. The collected germplasm (27 accessions) were germinated and evaluated in both laboratory and nursery at ICAR-CAZRI, RRS, Kukma-Bhuj, Gujarat. Data were recorded on its germination and growth parameters. Statistical analysis revealed the presence of high coefficient of variation for morphological parameters. Range of variation for important traits of *G. tenax viz.*, plant height (cm), number of fruits per 100 g, 1000-seed weight (g) and fruit thickness (mm) were observed with range (20.8 to 78.5, 690 to 1800, 60.3 to 150.7 and 4.0 to 6.7) and mean (46.8, 971.6, 113.8 and 5.1) value, respectively. Range of value of Pearson's correlation coefficient varied from – 0.85 between NF and TSW to 0.83 between PL and leaf width. Cluster analysis revealed the presence of three major clusters at coefficient value of Euclidian distance of 6.0. PCA analysis revealed the presence of 75.59% variability among *G. tenax* genotypes through first five most informative components and it also confirmed the clustering pattern obtained through cluster analysis.

Key Words: Cluster analysis, Correlation, Genetic diversity, *Grewia tenax*, PCA analysis

Introduction

Grewia tenax popularly called white cross-berry is most common in arid and semi-arid plains, lowlands and lower mountains up to a maximum altitude of 1,250 m a.s.l. and in regions with mean annual rainfall of 200-1000 mm in sandy, rocky and lateritic soils. It is widely distributed in the lowlands and mountains of Arabian countries, Egypt, Iran, Afghanistan, Sudan, Pakistan, Srilanka and India (Alrikain, 2004; Gebauer *et al.,* 2007; Sohail, 2009). In India, wild genotypes of *G. tenax* are commonly grown naturally in rocky and gravelly piedmont plains of arid and semi-arid areas of Gujarat, Rajasthan, Andhra Pradesh, Haryana, Karnataka, Maharashtra and Punjab (Sharma and Patni, 2012; BSI, 2014). In India about 120.4 mha area has been classified as degraded and wasteland category, of which major part lies in western part of Rajasthan, and Kachchh and Saurashtra regions of Gujarat. About 16% (3,129 thousand ha) of total geographical area (19,683 thousand ha) of Gujarat state is under degradation. As Tropic of Cancer passes through the Rann of Kachchh, Gujarat, the region experiences intensely hot and arid climate (Priyanka *et al.,* 2015). These areas are enriched with thorny forest, which include species of trees, shrubs and herbs. The important under-utilized shrubs of this region are *Acacia senegal* (Mimosaceae), *Commiphora wightii* (Burseraceae) *Euphorbia caducifolia* (Euphorbiaceae), *Grewia villosa* (Tiliaceae), *Salvadora oleoides* and *S. persica* (Salvadoraceae), *Premna resinosa* (Verbenaceae), and *Ziziphus nummularia* (Rhamnaceae). Among these, *G. tenax* has much potential value as food, fodder and medicine, yet to be exploited/utilized.

G. tenax is a typical tropical plant species which can tolerate seasonal drought and withstand temperatures of more than 50°C (Gebauer *et al.,* 2007). The plant is not only adapted to high temperatures, salinity and dry conditions, but also has deep roots which can stabilize sand dunes (Saied *et al.,* 2007). It is closely related to Phalsa (*G. asiatica*), popularly eaten fruit in North India. *G. tenax* has often been cited as a prime candidate plant for domestication as a useful horticultural plant (Gebauer *et al.,* 2007). It plays an effective role in rehabilitation of wastelands (Teketay, 1996) and requires only 200 mm of annual rainfall. Being tolerant to drought and soil salinity, *G. tenax* perform well in Kachchh region of Gujarat where due to arid climate that poses a major problems in establishment of any species in this region. Hence, this paper is an attempt to assess the genetic diversity in *G. tenax* on basis of morphological and pomological characteristics, and it would also help in developing adequate phenotypic markers for identification of promising genotypes and germplasm management.

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Materials and Methods

Experimental Site

The location of the study area was nursery and farm area of ICAR-CAZRI (Central Arid Zone Research Institute), Regional Research Station, Kukma-Bhuj, Gujarat (India) (latitude 23°21'19" - 23°21'33" N, longitude 69°79'72" - 69°78'78"E). The climate of the Kachchh region is hot arid with a maximum temperature of 48°C during summer and minimum 2°C during winter. The average rainfall of the region is 326 mm and most of rainy days occur during July to September with high evapo-transpiration (1500-2000 mm per year).

Field Survey and Collection

Field surveys were conducted in arid region of Kachchh, Gujarat for the occurrence of *G. tenax* during the months of March to October 2015. The 27 germplasm accessions of *G. tenax* as fruits collected from different sites were brought to laboratory and morphological parameters of 50 fruits from each accession such as fruit weight (g), diameter (mm), length (mm) etc. as well as qualitative traits *viz*., fruit morphology, colour and fruit lobe were measured. Length, width and diameter of fruits were measured with a digital Vernier caliper using method described by Gogu *et al.* (2009).

Nursery and Field Evaluation

The 27 germplasm accessions were evaluated in both laboratory and nursery. Germination studies were conducted by sowing seeds of collected accessions in polythene bags (size: 15 x 10 cm), filled with sand, soil and farm yard manure in 1:2:1 ratio. Seeds were also sown in portray having coco-peat, vermiculite and perlite (2:1:1 ratio) as supportive materials. Two seeds were sown in each bag and portrays compartment and kept in shade net house (50% sun light) and were irrigated with normal tap water daily until seeds germinated. Different growth parameters such as plant height (cm), number of branches per plant, stem girth (mm), leaf length (mm), leaf width (mm), petiole thickness (mm) were estimated at monthly interval in the nursery as well as in one year old plants at field.

Data Analysis

Data recorded were subjected to statistical analysis such as descriptive statistics, Pearson's correlation and multivariate analysis by using the software package "PAST3" (Hammer *et al.,* 2001).

Results and Discussion

Genetic diversity study of *G. tenax* genotypes collected from Gujarat arid zone were subjected to morphological and pomological characterization.

Range of Variation

Range of variation for 27 *G. tenax* genotypes (Table 1) revealed that maximum amount of variation present in the material. Coefficient of variation (CV) for quantitative traits such as DTG, GP, NL, NB, PH and PL was found to be 69.3, 67.0, 36.8, 35.4, 35.2 and 34.8 percent, respectively. Range of variation for important traits *viz.,* plant height (cm), leaf length (mm), number of fruits per 100 g, 1000-test weight (g) and fruit thickness (mm) was observed with range (20.8 to 78.5, 21.4 to 55.9, 690 to 1800, 60.3 to 150.7 and 4.0 to 6.7) and mean (46.8, 35.1, 971.6, 113.8 and 5.1) value, respectively. The maximum number of fruits per 100 g was obtained on genotype CZBGT-3 (1800) followed by CZBGT-6 (1573.33) and CZBGT-31 (1305), whereas minimum was recorded in CZBGT-10 (690). Maximum 1000-test weight (g) was observed in germplasm CZBGT-19 (150.7), closely followed by CZBGT-1 (150.0) and CZBGT-29 (146.7), while minimum was found in CZBGT-3 (60.3). Understanding of nature and amount of variability present in genotypes of crops might be useful in improvement of traits of existing germplasm which could be used in breeding programs of that crop. Furthermore, this kind of evaluation will provide information about uniqueness and distinctness of genotypes, which is of vital importance in optimal and effective conservation of genotypic variability (Bisrat *et al.,* 2000). Genetic diversity is best estimated if agro-morphological, biochemical and marker studies are used together (Lattoo *et al.,* 2008).

Pearson's Correlation Analysis

Correlation analysis is an important statistical tool which provides the relationship between two random variables whether they are dependant or independent. Pearson's correlation matrices of quantitative traits of 27 *G. tenax* genotypes are presented in Table 2. Range of value of correlation coefficient varied from –0.85 between number of fruits per 100 g and TSW to 0.83 between petiole length and leaf width.

Cluster Analysis

Dendrogram was generated for 14 quantitative traits of 27 *G. tenax* genotypes by using Euclidian distance

based UPGMA method of cluster analysis (Fig. 1). The coefficient of Euclidean genetic distance ranged from 0.0 to 6.2, which indicated the presence of considerable amount of genetic variability among *G. tenax* genotypes. It formed three major clusters at coefficient value of Euclidian distance of 6.0 with two sub-clusters each, excluding third cluster, where genotype CZBGT-31 grouped alone. In general, the pomological traits viz., petiole length, petiole thickness, fruit length, thickness, width and thousand seed weight played a major role

Table 1. Range of variation in plant and fruit characteristic of 27 *Grewia tenax* genotypes collected from arid Kachchh of Gujarat

Traits	Min.	Max.	Mean	SE (±)	SD	Skewness	Kurtosis	CV (%)
Day to germination	13.7	99.3	31.0	4.1	21.5	1.8	3.2	69.3
Germination %	0.0	45.0	15.0	1.9	10.1	1.3	1.9	67.0
Plant height (cm)	20.8	78.5	46.8	3.2	16.4	0.4	-0.8	35.2
No. of branches	2.0	9.0	4.0	0.3	1.4	1.6	4.7	35.4
Stem girth (mm)	1.5	7.9	4.5	0.3	1.5	0.0	-0.1	33.2
No. of leaves	17.3	87.7	37.8	2.7	13.9	1.8	5.5	36.8
Leaf length (mm)	21.4	55.9	35.1	1.5	7.7	1.3	2.3	22.0
Leaf width (mm)	20.9	61.6	34.3	1.8	9.2	1.6	3.3	26.8
Petiole length (mm)	6.1	23.9	12.2	0.8	4.2	1.2	1.5	34.8
Petiole thickness (mm)	0.4	1.3	0.8	0.0	0.2	1.0	0.9	26.0
No. of fruits /100 (g)	690.0	1800.0	971.6	49.7	258.3	1.8	3.6	26.6
1000-seed weight (g)	60.3	150.7	113.8	5.0	25.9	-0.3	-0.9	22.7
Fruit thickness (mm)	4.0	6.7	5.1	0.1	0.6	0.1	1.0	11.3
Fruit length (mm)	5.5	12.0	7.6	0.4	2.1	0.7	-1.2	27.1
Fruit width (mm)	5.0	13.3	8.9	0.4	2.1	0.0	-0.6	23.4

Table 2. Pearson's correlation coefficient for quantitative traits of 27 *Grewia tenax* genotypes

Traits	DTG	GP	PH	NB	SG	NL	LL	LW	PL	PT	NF	TSW	FrT	FrL
Day to germination (DTG)														
Germination % (GP)	0.16													
Plant height (cm) (PH)	-0.31	-0.09												
No. of branches (NB)	-0.08	-0.28	-0.29											
Stem girth (mm) (SG)	-0.40*	0.06	-0.14	0.27										
No. of leaves (NL)	-0.34	0.06	0.13	0.13	0.59**									
Leaf length (mm) (LL)	-0.40*	0.22	0.15	0.05	0.39*	0.33								
Leaf width (mm) (LW)	-0.21	0.24	0.00	0.10	0.45*	0.36	0.50**							
Petiole length (mm) (PL)	-0.29	0.18	0.02	0.05	0.51**	0.52**	0.39*	0.83**						
Petiole thickness (mm) (PT)	-0.14	0.31	0.30	-0.26	0.08	-0.07	0.35	0.30	0.01					
No. of fruit/100 (g) (NF)	0.03	-0.03	-0.22	0.13	-0.30	-0.32	0.06	-0.12	-0.24	-0.23				
1000-seed weight (g) (TW)	-0.15	0.03	0.24	-0.02	0.48*	0.40*	0.14	0.21	0.30	0.12	-0.85**			
Fruit thickness (mm) (FrT)	-0.09	0.03	0.17	-0.22	0.02	0.15	-0.02	0.21	-0.07	0.30	-0.27	0.23		
Fruit length (mm) (FrL)	-0.29	0.03	0.10	0.17	0.43*	0.45*	0.50**	0.42*	0.71**	-0.09	-0.15	0.21	-0.45*	
Fruit width (mm) (FRW)	0.16	-0.01	0.01	-0.31	-0.25	-0.30	-0.22	-0.22	-0.50**	0.25	-0.17	0.15	0.55**	-0.81**

*Significant at $p < 0.05$ / **Significant at $p < 0.01$

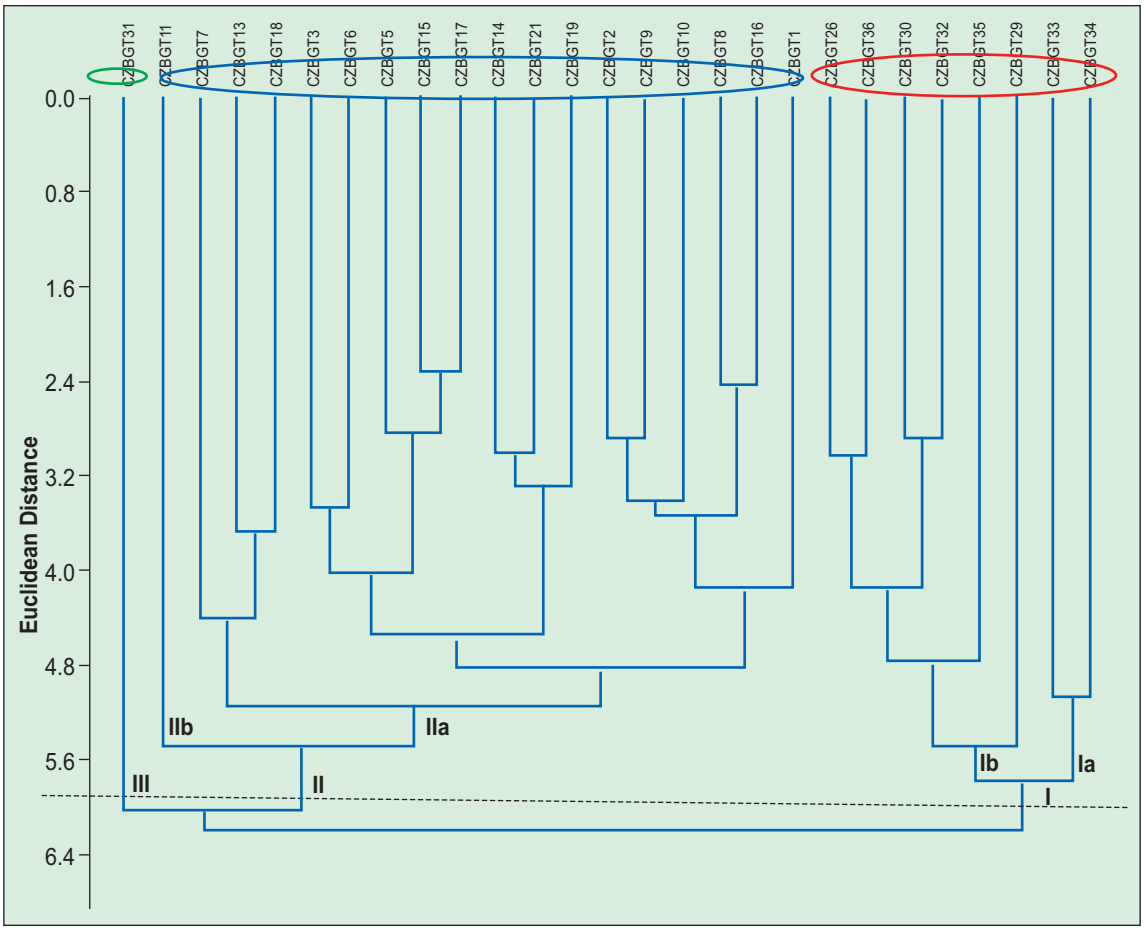

Fig. 1. Dendrogram of 27 *Grewia tenax* genotypes based on UPGMA method of cluster analysis using Euclidian distance

in grouping of genotypes into different clusters. At coefficient value of Euclidian distance of 5.6, Cluster-I formed two sub-clusters Ia and Ib with two (CZBGT-34 and CZBGT-33) and six (CZBGT-29, CZBGT-35, CZBGT-32, CZBGT-30, CZBGT-36 and CZBGT-26) genotypes, respectively. These genotypes exhibited better in length and thickness of petiole and fruit than other genotypes. At coefficient value of Euclidian distance of 5.4, Cluster-II formed two sub-clusters named IIa and IIb with 17 and single (CZBGT-11) genotypes, respectively. These genotypes showed comparatively low in length and thickness of petiole and fruit to differentiate from genotypes under Cluster-I. Further, the genotype present in Cluster-III, CZBGT-31 showed intermediate in above characters, which clearly separated it from Cluster-I and II. This kind of grouping pattern on basis of pomological traits were reported in cluster analysis in horticultural crops viz., Apricot (Mratinic *et al.*, 2011; Kumar *et al.*, 2015), turmeric (Bahadur and Meena, 2016) and wild hip rose (Verma *et al.*, 2015). The pairs of genotypes present in sub-cluster, Ib [(CZBGT-30 and CZBGT-32); (CZBGT-26 and CZBGT-36)] and in sub-cluster, IIa (CZBGT-15 and CZBGT-17; CZBGT-5), (CZBGT-8 and CZBGT-16), (CZBGT-2 and CZBGT-9) and (CZBGT-14 and CZBGT-21; CZBGT-19) showed least variability between them. The maximum genetic distance (8.96) observed between genotypes, CZBGT-14 and CZBGT-34 followed by CZBGT-34 and CZBGT-13 (8.53) on basis of Euclidean distance, whereas, minimum between CZBGT-15 and CZBGT-17 (2.32).

Principal Components Analysis

The PCA analysis performed on 14 quantitative traits of 27 *G. tenax* genotypes revealed that the first five most informative components accounted for 75.79% variance (Table 3). Almost, similar amount of variability revealed in PCA analysis study on Apricot with > 80% (Mratinic *et al.*, 2011) and 73.44% (Kumar *et al.*, 2015) in first 4 and 3 PC components and 85% in fist 4 PC components in wild hip rose (Verma *et al.*, 2015). The variability contributed by PC axis-I was 28.81% followed by PC axis-II (18.21%), III (11.34%), IV (9.12%) and V

Table 3. The cumulative % variance of first five Principal components obtained through PCA analysis of 27 *Grewia tenax* genotypes

Principal component	% Variance	Cumulative % Variance
I	28.81	28.81
II	18.20	47.01
III	11.34	58.35
IV	9.12	67.47
V	8.32	75.79

(8.32%). The characters such as petiole length (0.40), fruit length (0.38), stem girth (0.35), number of leaves (0.34), leaf width (0.34), leaf length (0.30) and thousand seed weight (0.23) were major contributors for 28.81% variability of PC axis-I. The fruit thickness (0.45), fruit width (0.42), petiole thickness (0.36) and thousand seed weight (0.33) were responsible for 18.21% variability in PC axis-II, whereas, the characters such as germination percentage (0.42), petiole thickness (0.41), number of fruits (0.38) and leaf length (0.35) were accountable for 11.34% of variability in PC axis-III. In general, the pattern obtained through cluster analysis confirmed by two-dimensional scatter plot of PCA ordination using the first two most informative PC axes obtained in the present study through PCA analysis (Fig. 2). Nevertheless, several years of studies must be conducted before parental selection for a possible plant breeding. The PCA analysis provided a simplified classification of the *G. tenax* genotypes for collecting and improvement. This scatter plot also shows geometrical distances among genotypes that reflects similarity among them in terms of traits measured.

The morphological and pomological diversity study on 27 genotypes revealed the presence of considerable amount of genetic variability among them. Multivariate analysis was found useful for detection of phenotypic and pomological differences among the *G. tenax* genotypes. Principal component analysis revealed that traits related to petiole length, fruit length, fruit thickness, number of fruits, thousand seed weight, stem girth, leaf length and width accounted for distinct variability among different genotypes. Identification and description of the genetic variability in *G. tenax* genotypes are preliminary requirements for the exploitation of its useful traits in

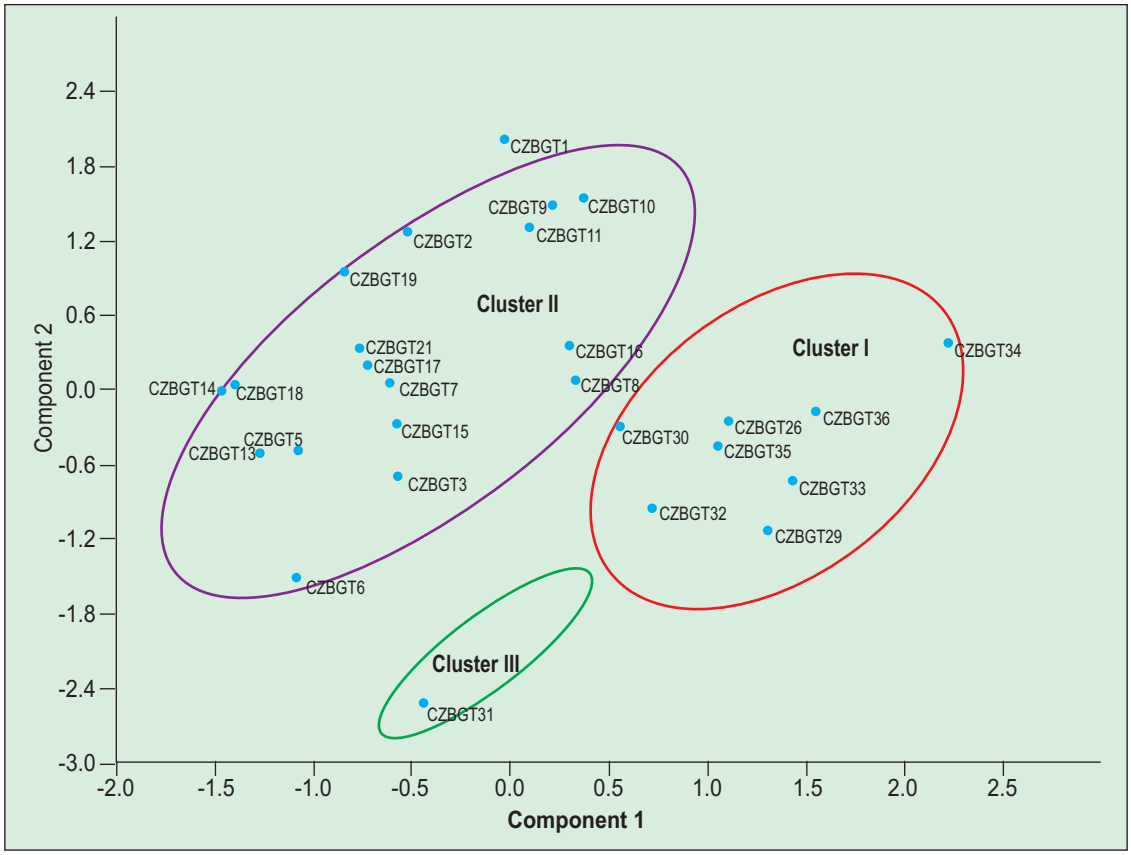

Fig. 2. Two-dimensional scatter plot of PCA ordination using the first two most informative PC axes obtained for 27 *Grewia tenax* genotypes through PCA analysis

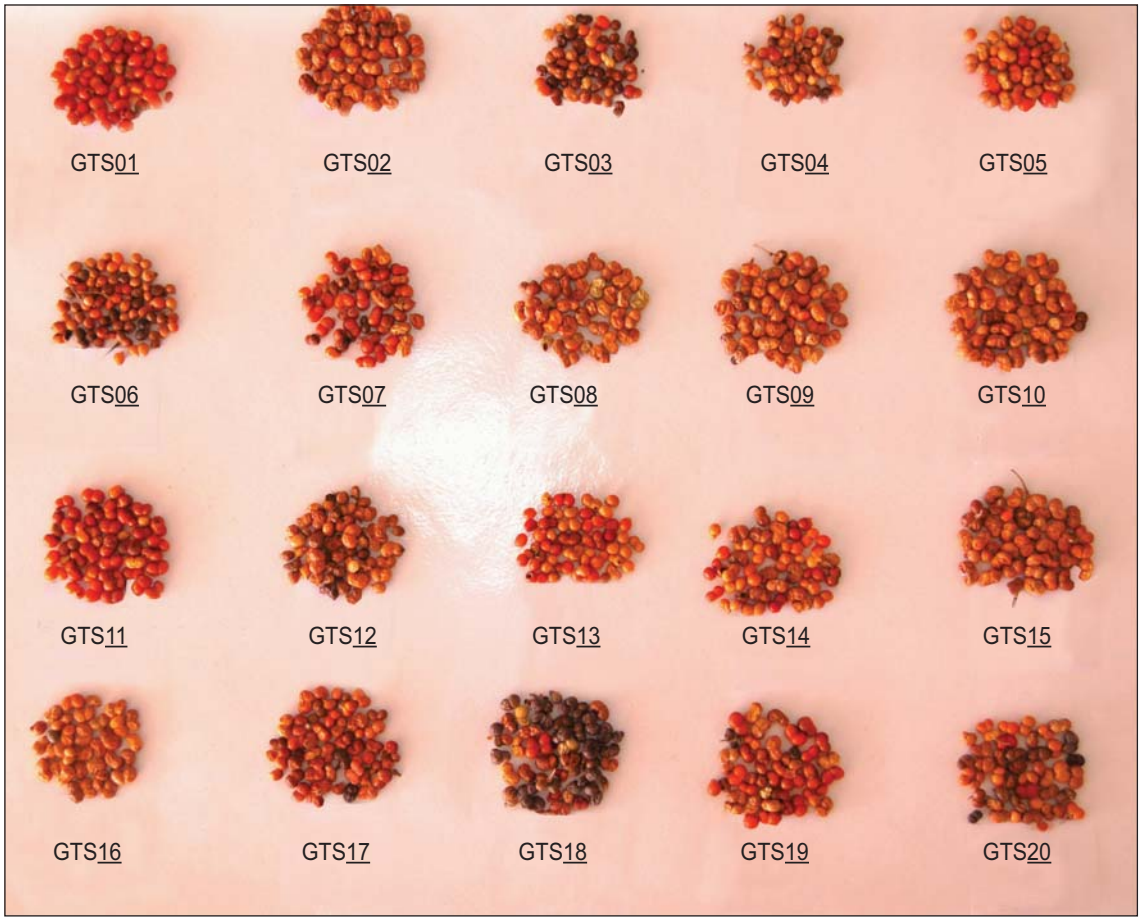

Fig 3. Morphological variation in collected *Grewia tenax* seeds

introducing them into Alternate Land Use system and into rangelands of hot arid regions of India for food and fodder security of the rural inhabitants.

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