Research Article

Biodiversity Evaluation among Wild Jujube (*Ziziphus nummularia* (Burm. F.) Population in Thal Desert of Pakistan

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Abstract | Thal zone of Pakistan is massive treasure of wild jujube germplasm (*Ziziphus nummularia* (Burm. F.)), but currently due to deforestation, global climatic changing scenario and shifting attitude of farmers towards other cash crops, the specie is in high risk of extinction. In this study, sixteen naturally growing wild jujube accessions were analysed based on multivariate analysis. Significant diversity was counted for selected morphological and biochemical traits like leaf length (1.8-5.4cm), thorn length (0.6-2.9 cm), fruit weight (1.88-4.72g), fruit length (8.83-19.34mm), fruit width (11.03 -22.74mm), stone weight (0.32-1.09g), TSS (5.9-13.2%) and vitamin C contents (131.2 to 165.56mg/100g). Most positive correlation was noted between leaf length and leaf width (r=0.897) whereas, the correlation between stem girth and stone width (r= -0.409) was most negative. In addition, principal component analysis (PCA) made it possible to establish similar and dissimilar groups of accessions depending on investigated traits. Dendrogram was successfully constructed with two main clusters (C1, C2) which further partitioned into sub clusters i.e. C1A, C1B, C2A and C2B. Fruit colour meter value showed average +a* =2.7 and average +b* =13.0 for Bhakkar accessions, while for Layyah accessions these were 3.8 and 16.4, respectively. Such variation can strengthen jujube germplasm conservation, be able to provide strong basis for initiating conventional breeding programmes and can be helpful in biotechnology for gene transfer process. Management of natural plantation of *Ziziphus nummularia* in Thal zone is highly favoured to save rich genetic resources of this unique jujube specie.

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1. Introduction

Natural resources of perennial plants existing in arid climate provide good basis for the selection and development of novel gene pool (Sudhersan and Ashkanani, 2009; Bal, 2013). About 80% area of Pakistan lies in arid to semi-arid regions receiving less than 250 mm unpredictable annual rainfall (Shah *et al*., 2011; Baig *et al*., 1999; Farooq *et al*., 2009) on which *Z. nummularia*is grown naturally as native plant. This perennial shrub is of high attention to rehabilitate degraded lands, protect soil erosion and widely utilized as food by rural people as well as for root-stock material to produce superior quality jujube plants (Laamouri *et al*., 2008; Maraghni *et al*., 2010; Oliet *et al*., 2012). Wild jujube stones have been gathered on Deccan plateau dated to 3500-3000BC before Gangetic civilization (Pareek, 2001). It is indigenous to Southern China,Malaysia,Afghanistan, North Africa and Australia but is primarily confined to India, Pakistan, Iran and Saudi Arabia (Obeed *et al*., 2008).

Ziziphus nummularia is also considered as progenitor of cultivated jujube, so its gene pool is also important for genetic improvement of domesticated jujube. Also, it is an imperative source of manyphyto-chemical components, nutrients and natural bio-active substances (Tirado and Pugnaire, 2005; Wojdylo *et al*., 2016), which manifest the needs to exploit this neglected specie for natural resources conservation, poverty alleviation and to expand farmer's livelihood (Pandey *et al*., 2010; Gupta and Anil, 2014). Furthermore, extracts from its different tree parts are being used in folk medicines to relieve the effects of different chronic health problems i.e. insomnia, skin diseases, inflammatory conditions and fever (Singh *et al*., 2006; Abdeddaim *et al*., 2014; Hammia *et al*., 2015; Rais *et al*., 2017). Wild jujube species is botanically a highly branched shrub with ovate to orbicular leaves and paired stipular spines. Edible fruit is golden yellow to dark brown at maturity and possessing high mucilaginous pulp property with elevated ascorbic acid contents up to 183.42 mg/100 g (Rathore, 2009; Wang *et al*., 2016).

Ziziphus are among those species which are at high risk of extinction due to over grazing, urbanization, deforestation and lack of conservation and cultivation practices (Hedrick and Kalinowski, 2000; Wang *et al*., 2012: Zhang *et al*., 2015). Exploration of resistant genotypes with promising fruit properties among local cultivars is basic step for breeding programs (Ghazaeian, 2015; Tatari *et al*., 2016). Various techniques used to analyze diversity include morphological markers, cytological markers, biochemical markers and molecular markers. Measures of genetic diversity cuddled with coefficient of parentage, genetic and allelic diversity. Latest statistical tools used to measure diversity are metroglyph analysis, D2 statistics, principal component analysis (PCA), principal coordinate analysis (PCoA), canonical analysis, factor analysis and correspondence analysis (Bi, 2015). However, morphological approaches are simple, cheap, direct, inexpensive and easy to use by researcher (Zhang *et al*., 2015). Wild genotypes, however, do not have acceptable yield so that they could intrigue breeders, but still are valuable resources to improve resistance to biotic or abiotic stresses and nutritional quality (Ahmed *et al*., 2016; Singh *et al*., 2014).

In fact, investigating the genetic diversity is a foremost step towards conserving the genetic resources and the resulting information might provide the stakeholders with some management strategies (Pollard *et al*.,2002). However, little information regarding wild jujube diversity across the Pakistan arid zones is available. Genetic variability in wild jujube is introduced by its dominantly cross-pollinating nature along with natural seed germination, which can be exploited for selection and genetic improvement (Singh *et al*., 2006).

Climatic conditions of Thal zone (Layyah and Bhakkar) are quite suitable for wild jujube and the area is natural trove for this wild jujube specie. Fruit is mainly consumed by children and rural community of the area as fresh and dried both. Recently issues like deforestation, over grazing, global climatic changes and farmers negligence towards this highly nutritious arid fruit, it needs to exploit and evaluate. Morphological characterization is basic step towards diversity evaluation, to expand the gene pool and conserve the genetic resources whereas the biochemical traits assessment is mandatory to elevate the nutritional potential. Aims of this study include (i) characterization of wild gene pool from Thal zone (Layyah and Bhakkar) (ii) evaluation based on morphological, biochemical and color meter attributes (iii) accessions with good morpho-biochemical traits can be selected to expand the existing jujube gene pool in country. The main hypothesis of this study is to recognize the promising potential of wild jujube (*Ziziphus nummularia*) growing in Thal zone due to its excellent properties like xerophytic nature with excellent nutritional and medicinal properties. This research will also identify the traits to develop reliable germplasm identification key for assisting in variety registration programs.

2. Materials and Methods

2.1 Plant material

Sixteen wild growing jujube accessions were selected from the Thal desert of Pakistan i.e. Layyah and Bhakkar districts situated between the Indus and Chenab rivers along with Sindh Sagar Doab with geographical coordinates 30.96°N, 70.94°E and 31.60° N, 71.08°E. Fruit samples were collected during the month of March. The area is characterized by long and sweltering summer. Germplasm was selected on visionary differences and single tree was considered as one accession. Key phenological growth stages for jujube in Thal area are; leaf emergence (May-June), bud development (July), flowering (September-October), fruit development (December-February) and fruit maturity (March-April).

Abbreviations of collected accessions along with their geographical coordinates and soil properties of Thal area are given in Tables 1 and 2, whereas coding of morphological quantitative traits are presented in Table 3. The selected jujube accessions were natural and sexually grown trees under arid climatic conditions. Map of selected sites (Thal zone) along with metrological conditions is shown in Figures 1 and 2, respectively whereas phenotypic features of fruits and leaves are exhibited in Figures 3 and 4.

2.2 Morphological traits evaluation

A total of thirty-four morphological traits (including eleven quantitative and eighteen qualitative) were measured to assess the diversity. Each individual accession was considered as treatment and thirty mature fruits of uniform shape, devoid of any disease symptom or insect pest attack were randomly collected to record data. Similarly, thirty leaf samples of uniform size were collected to determine leaf traits. Thirty thorns were selected around the tree canopy to record thorn traits. Relevant data for leaf, fruit and stone (including weight, length and width) was recorded by digital Vernier caliper (Model: HT1406-A1, China) provided a precision of 0.01 mm and digital weighing balance (Model UniB1C. SHIMADZU, U x 320g, Min.0.02g, e=0.01g and d=0.001g).

Table 1: Detail of 16 wild jujube (*Ziziphus nummularia*) accessions of Thal desert.

Sr. No.	Accession name	Accession ID	Collection site	Latitude (°N)	Longitude (°E)	Altitude (ft)
1	Bhakkar 1	BKR 1	Bhakkar	31.95°	71.1°	486
2	Bhakkar 2	BKR 2	Bhakkar	31.43°	71.19°	560
3	Bhakkar 3	BKR 3	Bhakkar	31.62	71.06	572
4	Bhakkar 4	BKR 4	Bhakkar	31.55	71.23	570
5	Bhakkar 5	BKR 5	Bhakkar	31.72	71.42	548
6	Bhakkar 6	BKR 6	Bhakkar	31.81	71.39	567
7	Bhakkar 7	BKR 7	Bhakkar	31.45	71.45	531
8	Bhakkar 8	BKR 8	Bhakkar	31.30	71.4	581
9	Bhakkar 9	BKR 9	Bhakkar	31.22	71.8	582
10	Bhakkar 10	BKR10	Bhakkar	31.17	71.5	583
11	Layyah 11	LYH 11	Layyah	30.97	70.94	470
12	Layyah 12	LYH 12	Layyah	30.73	70.83	462
13	Layyah 13	LYH 13	Layyah	30.57	70.52	439
14	Layyah 14	LYH 14	Layyah	31.19	71.05	458
15	Layyah 15	LYH 15	Layyah	30.58	70.52	453
16	Layyah 16	LYH 16	Layyah	30.12	71.05	454

Table 2: Soil profile of Thal desert (Layyah and Bhakkar).

Soil characteristics	Layyah	Bhakkar
Soil texture	Sandy loam	Sandy loam
pH	8.3	8.1
OM%	0.70	0.84
$CaCO_3$	4.2	5.30
EC (dS/m)	0.94	0.96
Saturation %	31	30
Phosphorus (mg/Kg)	6.8	9.3
Potassium (mg/Kg)	100	86
Zinc (mg/Kg)	0.54	0.62
Copper (mg/Kg)	0.18	0.17
Iron (mg/Kg)	3.10	4.9
Manganese (mg/Kg)	0.75	0.86
Boron (mg/Kg)	0.41	0.39

2.3 Biochemical traits evaluation

Fruits sampled for morphological traits estimation were used for biochemical traits (total soluble solids (%), fruit acidity (%), vitamin C (mg/100g), reducing sugars (%), non-reducing sugars (%), total sugars (%) and total phenolic contents (μg GAE mL^{-1})) analyses. The total soluble solids were quantified by use of digital refrectometer (RX5000, ATAGO, Japan). Fruit acidity and vitamin C contents was measured by following Hortwitz (1960) and Ruck (1969) respectively whereas sugars (reducing, non-reducing and total) were measured by considering Hortwitz (1960) and Ronald and Sawyer (1981). Total phenolic contents (µg GAE mL^{-1}) were assessed with the method of Ozgen *et al*. (2010).

Table 3: Coding of morphological quantitative characters used for evaluating 16 wild jujube accessions of Thal desert.

Quantitative characters	Unit	Code
Stem girth	ft	Stmgr
Leaf length	cm	Lflnt
Leaf width	cm	Lfwid
Petiole length	cm	Ptlent
Thorn length	cm	Thlent
Fruit weight	g	Frtwt
Fruit length	mm	Frtlent
Fruit width	mm	Frtwdt
Stone weight	g	Stwght
Stone length	mm	Stlent
Stone width	mm	Stwdth

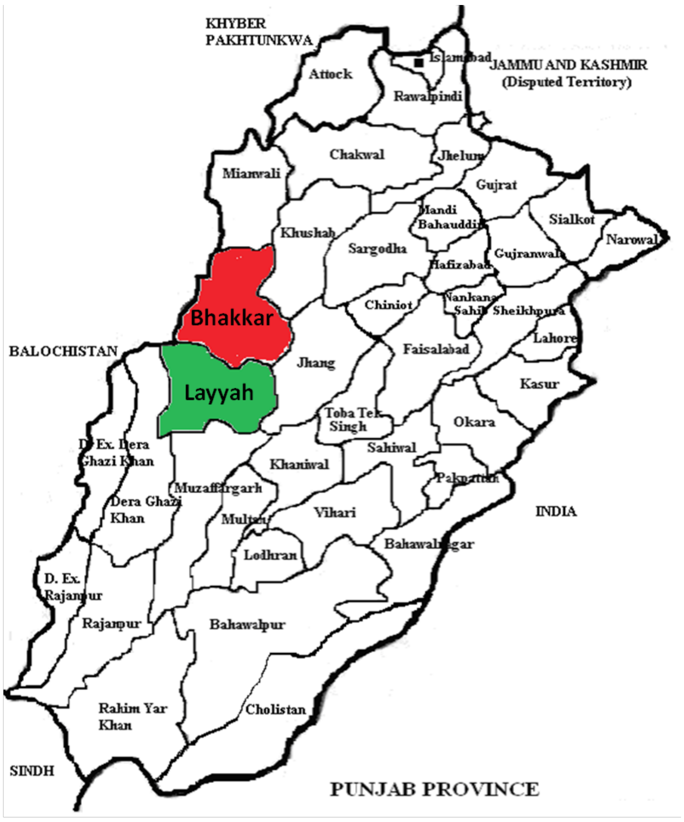

Figure 1: Map of Punjab province (Pakistan). Colored areas (red and green) are showing the collection regions of 16 wild jujube accessions from Thal desert.

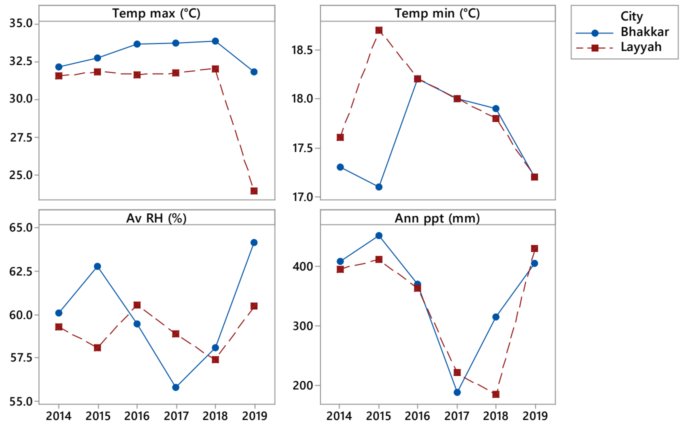

Figure 2: Meteorological parameters of germplasm collection sites (2014 to 2019): Tmax: Average annual maximum temperature (°C), Tmin: Average annual minimum temperature (°C), Av. RH: Average annual humidity (%), Ann pp: Annual total precipitation (mm). Source: Regional Metrological Centre, Lahore, Pakistan.

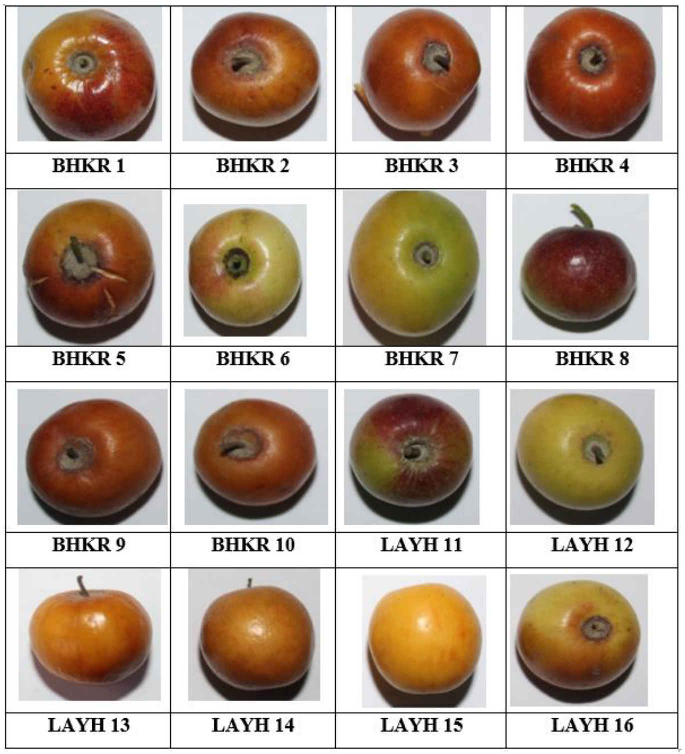

Figure 3: Fruits phenotypic features of wild jujube germplasm selected from Thal desert.

2.4 Fruit color evaluation

Fruit color of selected accessions was measured by using the colorimeter (CR-400 Minolta). It was made by using the head 15mm in diameter of the Hunter Color lab and recorded in CIELAB units of L*, a* and b*. The Hunter color lab was calibrated utilizing the manufacturer's standard black and white tiles. L*a*b* color space indicates different degrees of color measurement, in hue which L* value indicates the lightness (black (L^*=0) and (L^* = 100)), a^* value indicates redness-greenness (red (a^* = 100) and green (a^* = -100)) and b^* indicates yellowness-blueness (yellow (b^*= 100) and blue (b^* = -100)). Both chroma and hue were derived from $a^*$and b^* using the equations: chroma (C = (a^*) 2 + (b^*) 2)1/2) and angle (h = arc tan (b^*/a^*)) (Varakumare *et al*., 2011). The color coordinates showed the variation between the basic colors of different wild jujube accessions. Thirty respective fruits (already used for morphological and biochemical traits evaluation) from each accession were analyzed. The colorimeter was set to enable the light pulse to move around three positions of each fruit surface for making precise measurement.

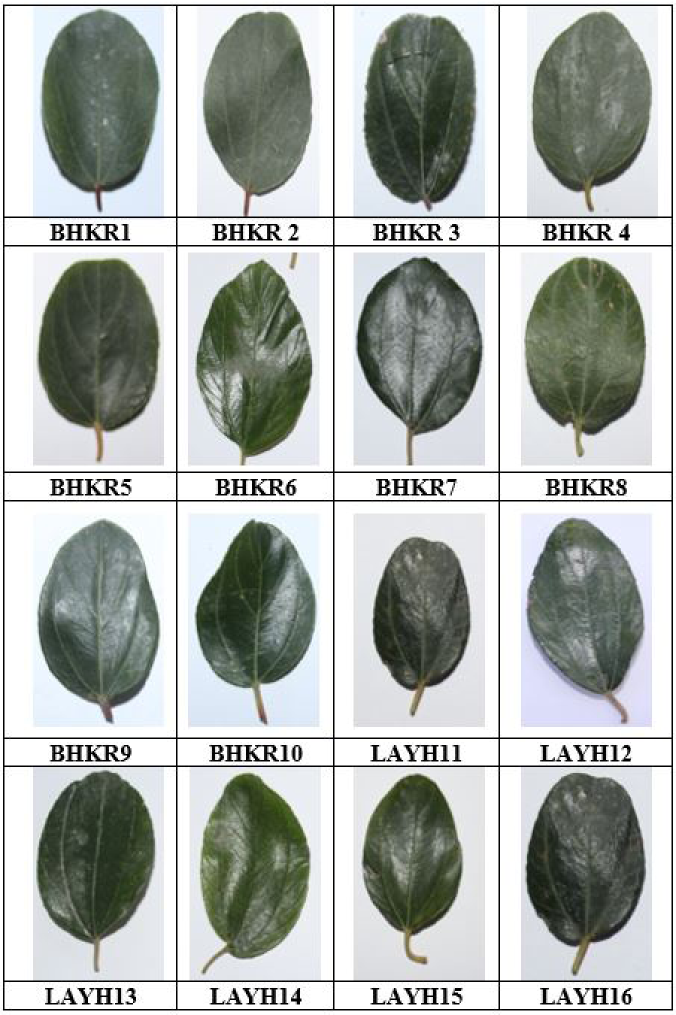

Figure 4: Leaf diversity in wild jujube germplasm selected from Thal desert.

2.5 Data scoring and analysis

Data generated from 16 wild jujube accessions associating to 11 morphological quantitative traits was analyzed by following XLSTAT (2018) software. The coefficient of variation (%) was calculated to determine the existing variability. Correlation coefficients were evaluated to select useful characters for efficient indirect selection and to run down ineffective traits. Genetic similarity was counted and constructed relevant PCA plots. Morpho-qualitative traits were estimated by considering jujube descriptor (NBPGR, 2002) with few modifications. Dendrogram was assembled by utilizing joint data from morphological quantitative and qualitative traits. Euclidean distance was used in Ward's method for agglomerative hierarchical clustering (AHC).

Biochemical characters were analyzed as complete randomized design by considering each accession as a treatment. The analysis of variance (ANOVA) was conducted by using Statistix 8.1 to test the significance of variation between accessions for each biochemical traits. And significant mean differences ($p < 0.05$) were counted according to Tukey's test. Descriptive statistics are important to assess the fruit color variation among accessions and the CV is vital for variability index determination. So, to analyze color meter values descriptive statistics i.e. the values of minimum, maximum, mean and coefficient of variation (CV %) were computed.

3. Results and Discussion

3.1 Statistical indices

The descriptive statistics of minimum and maximum means, standard deviations and coefficient of variation (CV %) for eleven morphological quantitative traits are exhibited in Table 4. The results exhibited spacious morphological variability. Several traits like stem girth (38.80%), thorn length (35.46%), leaf width (33.51%), petiole length (31.88%), stone weight (31.86%), leaf length (29.56%) and fruit weight (24.59%) showed high CVs while the lowest CV was worked out for stone length (12.26%).

Stem girth ranged from 1.5 to 8.0 ft for BHKR4 and LAYH11, respectively. Leaf length diverged from 1.8 to 5.4 cm for LAYH 11 and LAYH 16. Leaf width varied 1.4 to 4.2 cm for BHKR3 and LAYH16, respectively. Petiole length was differentiated from1.2 to 3.6 cm for LAYH14 and BHKR2. Maximum thorn length (2.9 cm) was recorded for BHKR9, while minimum (0.6cm) for BHKR7. Fruit weight ranged 1.88-4.72g with the highest value in BHKR 7. Fruit length varied from 8.83 mm (LAYH 12) and 19.34 mm (LAYH 16). Fruit width was accounted from 11.03mm to 22.74mm with minimum in LAYH12 and maximum in BHKR1. Stone weight deviated from 0.32g (BHKR8) to 1.09g (BHKR5). Stone length (6.91 mm) and width (5.58 mm) was the lowest in BHKR4.

Table 4: Statistical indices for 11 morphological quantitative traits of 16 wild jujube accessions of Thal desert.

Variables	Minimum	Maximum	Mean	Std. deviation	CV%
Stem girth (ft)	1.500	8.000	3.919	1.521	38.80
Leaf length (cm)	1.800	5.400	2.800	0.828	29.56
Leaf width (cm)	1.400	4.200	2.050	0.687	33.51
Petiole length (cm)	1.200	3.600	2.250	0.717	31.88
Thorn length (cm)	0.600	2.900	1.638	0.581	35.46
Fruit weight (g)	1.880	4.720	3.426	0.843	24.59
Fruit length (mm)	8.830	19.340	15.403	2.861	18.57
Fruit width (mm)	11.030	22.740	16.688	3.474	20.81
Stone weight (g)	0.320	1.090	0.654	0.208	31.86
Stone length (mm)	6.910	11.820	10.039	1.232	12.26
Stone width (mm)	5.580	12.120	9.248	1.604	17.34

3.2 Correlations analysis

Strong positive correlation was detected among observed quantitative characters (Table 5). The strong positive correlation (0.897) was governed for leaf length and width. Other positive correlations were governed for fruit length and width (0.800), fruit weight and length (0.577), fruit weight and width (0.554), leaf width and stone weight (0.500), leaf length and stone weight (0.487), fruit width and stone weight (0.461), fruit length and stone weight (0.428). The most negative correlation was counted for stem girth and stone width (-0.409). Other negative correlations were governed for stem girth and stone length (-0.390), fruit length and stone width (-0.332), leaf width and stone width (-0.315), leaf length and stone width (-0.288), leaf length and thorn length (-0.259), fruit width and stone length (-0.256), thorn length and fruit width (-0.249), and stone weight and stone length (-0.241). Documentation of these traits may be supportive in selection of jujube accessions for future breeding programs to expand the jujube gene pool.

3.3 PCA analysis for morphological quantitative traits

Principal component analysis placed all quantitative traits into six components which showed 90.65% of total variation (Table 6). The first component which showed total variation of 31.979% included fruit length, leaf length, fruit width, leaf width, fruit weight, stone weight, stem girth and petiole length. Second component depicted 15.783% variability for stone width, stone length, fruit weight, stone weight, fruit width, thorn length, petiole length and fruit length. Third component constituted a total variability of about 13.633% for thorn length, stone weight, stem girth, fruit length, fruit width, and stone width. Forth factor shared a total variability of 13.126% shared by leaf width, leaf length, thorn length, stone weight and stone width. Total variability depicted by fifth factor was 9.735% by petiole length, stem girth, fruit weight, thorn length and stone weight. Sixth factor contributed a total variation of 6.40 % shared by stone length, fruit length, thorn length, fruit weight and stem girth.

Table 5: Correlation analysis among 11 morphological quantitative traits in 16 wild jujube accessions of Thal desert.

Variables	Stmgr	Lflnt	Lfwid	Ptlent	Thlent	Frtwt	Frtlent	Frtwdt	Stwght	Stlent	Stwdth
Stmgr	1										
Lflnt	0.064	1									
Lfwid	0.092	0.897	1								
Ptlent	0.093	0.084	0.222	1							
Thlent	0.056	-0.259	-0.114	-0.206	1	3					
Frtwt	0.112	0.287	0.296	0.39	-0.138	1					
Frtlent	0.253	0.333	0.22	0.042	-0.16	0.577	1				
Frtwdt	0.004	0.312	0.155	0.104	-0.249	0.554	0.800	1			
Stwght	0.285	0.487	0.500	-0.017	0.215	0.363	0.428	0.461	1		
Stlent	-0.390	-0.084	-0.117	0.068	0.059	0.031	-0.199	-0.256	-0.241	1	
Stwdth	-0.409	-0.288	-0.315	0	0.306	-0.021	-0.332	0.005	0.275	0.416	1

Values in bold are different from 0 with a significance level alpha=0.05. Abbreviations:Stmgr (stem girth), Lflnt (leaf length), Lfwid (leaf width), Ptlent (petiole length), Thlent (thorn length), Frtwt (fruit weight), Frtlent (fruit length), Frtwdt (fruit width), Stwght (stone weight), Stlent (stone lenth), Stwdth (stone width).

Table 6: First 6 components of the PCA analysis of 11 morphological quantitative traits of 16 wild jujube accessions of Thal desert.

Variables	F1	F2	F3	F4	F5	F6
Stem girth (ft)	0.342	-0.512	0.418	0.009	0.508	0.141
Leaf length (cm)	0.737	-0.023	-0.337	0.505	-0.202	0.028
Leaf width (cm)	0.690	-0.052	-0.339	0.610	0.020	0.013
Petiole length (cm)	0.252	0.137	-0.469	-0.209	0.721	-0.248
Thorn length (cm)	-0.274	0.250	0.606	0.399	0.262	0.289
Fruit weight (g)	0.664	0.379	-0.091	-0.316	0.269	0.201
Fruit length (mm)	0.780	0.064	0.222	-0.397	-0.171	0.292
Fruit width (mm)	0.720	0.306	0.178	-0.437	-0.275	-0.141
Stone weight (g)	0.649	0.366	0.445	0.390	0.055	-0.174
Stone length (mm)	-0.357	0.561	-0.436	0.049	0.036	0.525
Stone width (mm)	-0.354	0.837	0.177	0.102	0.054	-0.295
Variability (%)	31.979	15.783	13.633	13.126	9.735	6.400

Genetic diversity in each selected accession was also accessed by PCA analysis of first two components (Figure 5). Accessions close to the center of axis were considered less diverse and vice versa. Accession BHKR4 positioned in lower right plane and was found most diverse among all accessions. Factors involving behind this diversification might be the lowest stone length and width. LAYH16 was another diverse accession located on right plane with the largest leaf length and width and fruit length. BHKR5 was another varied accession placed in upper right plane having the largest stone weight and stone width. Accession BHKR9 and BHKR1 were clustered showing resemblance. LAYH 11 was placed in left upper coordinate and was far away from other accessions of this plane. BHKR3, BHKR10, BHKR8 and LYH 14 showed less diversity and were placed in lower left plane.

3.4 Variability among morphological qualitative traits

Considerable variation was counted for assessed 18 morphological qualitative characters as shown in Table 7. Tree shape was categorized as spreading, semi erect and erect. Branching habit was classified as drooping and semi drooping. Stem color was counted as light brown and brown. Leaf shape was counted as ovate and cordate, whereas leaf apex as obtuse or round. Foliage shape was as sparse or dense. Thorn arrangement was diverged as partial, caducous and persistent. Thorn shape was dispersed as all curved, alternate curved or straight. Most of the fruits were recorded with round fruit shape while some with oval or ovate shape. Fruit surface was as plain or ridged and wart. Stone shape was as round and oval with smooth and rough surface.

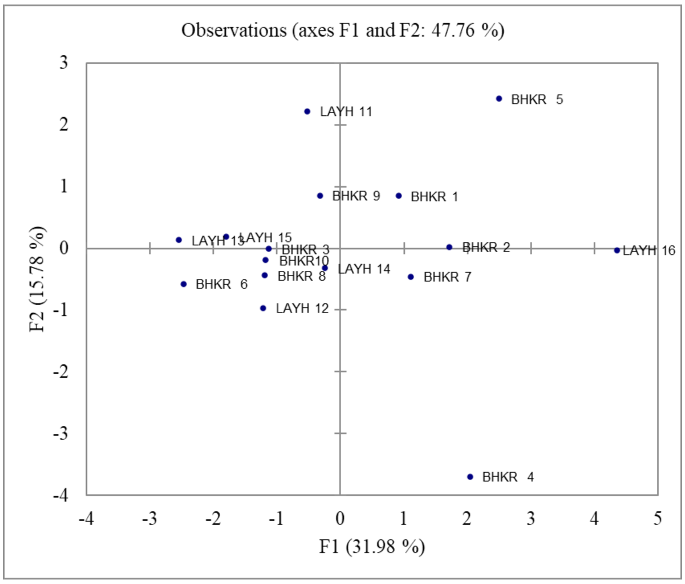

Figure 5: PCA plot based on the first two components for morphological quantitative traits of 16 wild jujube accessions of Thal desert.

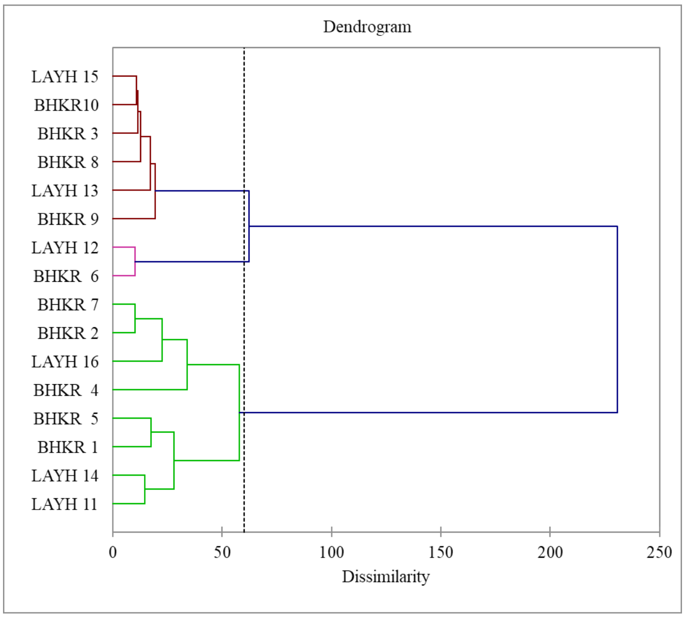

Figure 6: Dendrogram for 16 wild jujube accessions based on morphological traits.

3.5 Agglomerative hierarchical clustering (AHC)

Dendrogram with two major clusters (C1, C2) was effectively generated for investigated accessions. Cluster C1 comprised of eight accessions which were further partitioned into two sub clusters, i.e. C1A and C1B each containing four accessions. C1A was recognized with BHKR7, BHKR2, LAYH16 and BHKR4 and C1B had BHKR 5, BHKR1, LAYH14 and LAYH11. C2 further distributed into two clusters,

Table 7: Variability among qualitative traits of 16 wild jujube accessions of Thal desert.

Accessions	Tree shape	Branching habit	Stem color	Leaf shape	Leaf apex	Leaf base	Leaf margin	Foliage density	Thorn attachment	Thorn arrangement	Thorn shaoe	Fruit shape	Fruit apex	Fruit base	Fruit surface	Stone shape	Stone apex	Stone surface
BHKR 1	Spreading	Drooping	Light brown	Ovate	Obtuse	Acute	Crenate	Sparse	Persistent	Solitary	All curved	Round	Round	Round	Plain	Round	Round	Smooth
BHKR 2	Spreading	Drooping	Light brown	Cordate	Obtuse	Acute	Serrate	Sparse	Persistent	Solitary	All curved	Round	Round	Round	Ridged and wart	Round	Round	Smooth
BHKR 3	Spreading	Drooping	Brown	Cordate	Obtuse	Acute	Serrate	Sparse	Persistent	Paired	All curved	Round	Round	Round	Plain	Round	Round	Smooth
BHKR 4	Spreading	Drooping	Light brown	Ovate	Obtuse	Cordate	Crenate	Dense	Caducous	Paired	All curved	Round	Round	Pointed	Ridged and wart	Round	Round	Smooth
BHKR 5	Semi erect	Drooping	Light brown	Cordate	Obtuse	Acute	Serrate	Sparse	Persistent	Paired	All curved	Round	Round	Round	Plain	Round	Round	Rough
BHKR 6	Erect	Drooping	Light brown	Ovate	Round	Acute	Serrate	Dense	Persistent	Paired	All curved	Round	Flattened	Round	Plain	Round	Round	Smooth
BHKR 7	Spreading	Drooping	Light brown	Ovate	Obtuse	Cordate	Serrate	Sparse	Partial	paired	All curved	Round	Round	Round	Plain	Round	Round	Smooth
BHKR 8	Spreading	Semi drooping	Brown	Ovate	Obtuse	Acute	Serrate	Dense	Persistent	Paired	All curved	Round	Round	Pointed	Plain	Round	Round	Smooth
BHKR 9	Erect	Drooping	Light brown	Oval	Obtuse	Acute	serrate	Dense	Persistent	Paired	All curved	Round	Flattened	Round	Plain	Round	Round	Rough
BHKR10	Semi erect	Drooping	Light brown	Cordate	Obtuse	Acute	Serrate	Sparse	Persistent	Solitary	Alternate curved	Round	Round	Round	Ridged and wart	Round	Round	Smooth
LAYH 11	Semi erect	Drooping	Light brown	Ovate	Obtuse	Acute	Serrate	sparse	Persistent	Solitary	Alternate curved	Oval	Round	Flat	Plain	Oval	Obtuse	Smooth
LAYH 12	Erect	Semi drooping	Light brown	Ovate	Round	Acute	Serrate	dense	Persistent	Solitary	Alternate curved	Ovate	Round	Round	Plain	Oval	Round	Smooth
LAYH 13	Spreading	Semi drooping	Light brown	Ovate	Obtuse	Acute	Crenate	Sparse	Persistent	Paired	Straight	Ovate	round	Round	Plain	Oval	Obtuse	Smooth
LAYH 14	Erect	Semi drooping	Light brown	Oval	Round	Acute	Serrate	Sparse	Persistent	Paired	Straight	Round	Round	Round	Plain	Round	Round	Smooth
LAYH 15	Erect	Semi drooping	Light brown	Ovate	Obtuse	Acute	Serrate	Sparse	Persistent	Paired	All curved	Round	Round	Round	Plain	Round	Round	Smooth
LAYH 16	Erect	Semi drooping	Brown	Ovate	Obtuse	Acute	Serrate	Dense	Persistent	Paired	All curved	Round	Flattened	Round	Plain	Round	Round	Rough

Table 8: Mean values of biochemical traits of 16 wild jujube accessions of Thal desert.

Accessions	Total soluble solids (%)	Acidity (%)	Vitamin C (mg/100g)	Reducing sugars (%)	Non reducing sugars (%)	Total sugars (%)	Total phenolics (µg GAE mL^{-1})
BHKR 1	6.63±0.178 bcd	0.54± 0.018 cde	155.11±0.016 bc	8.37 ±0.129 h	9.74±0.107 def	18.12±0.078 ef	170.67±0.017 d
BHKR 2	5.83± 0.135 d	0.510± 0.039 de	143.36±0.013 f	9.917 ± 0.090 defgh	8.940 ±0.072 ef	18.957± 0.024 def	185.74± 0.016 bc
BHKR 3	7.86±0.165 bcd	0.676 ±0.051 bc	151.33± 0.012 bcd	13.160± 0.076 abc	3.153± 0.218 g	16.313±0.040 f	164.59± 0.012 d
BHKR 4	11.80±0.108 a	0.33± 0.090 fg	164.69±0.010 a	12.43±0.078 bcd	12.57±0.080 abc	25.00±0.079 ab	180.52 ±0.009 c
BHKR 5	7.33±0.122 bcd	0.476±0.084 def	133.55 ± 0.01 g	15.500± 0.038 a	8.25±0.053 f	23.75±0.041 abc	247.68± 0.007 a
BHKR 6	6.00±0.166 cd	0.670±0.083 bc	131.38± 0.01 g	13.62±0.066 ab	8.24±0.062 f	21.86±0.054 bcde	190.67±0.009 b
BHKR 7	9.20±0.115 ab	0.673±0.060 bc	147.75±0.013 def	9.147±0.069 fgh	10.470 ±0.049 cdef	19.617±0.043 def	140.48±0.011 e
BHKR 8	7.40±0.117 bcd	0.240±0.208 G	163.56±0.012 a	12.867±0.062 abc	13.66±0.064 a	26.52±0.059 a	163.96±0.021 d
BHKR 9	5.93±0.151 d	0.4167±0.0969 ef	149.68 ±0.019 cde	11.71± 0.139 bcdef	9.10±0.155 ef	20.81±0.014 cde	140.74±0.013 e
BHKR10	9.10± 0.111 abc	0.343±0.088 fg	156.38±0.017 b	11.40±0.072bc-defg	13.59±0.073 ab	24.99±0.033 ab	191.45±0.009 b
LAYH 11	6.30± 0.223 bcd	0.453±0.055 ef	143.17 ±0.015 f	12.167±0.069 bcde	9.840±0.064 cdef	22.007±0.0580 bcd	185.78±0.011bc
LAYH 12	6.33±0.091 bcd	0.540±0.055 cde	150.83±0.009 bcde	10.347 ± 0.065 cdefgh	11.413± 0.090 abcde	21.76±0.071 bcde	131.92± 0.028 f
LAYH 13	5.70±0.160 d	0.616±0.113 bcd	145.45±0.012 ef	9.473± 0.052 efgh	10.133±0.055 cdef	19.607±0.003 def	246.41±0.010 a
LAYH 14	6.83± 0.117 bcd	0.720±0.125 ab	148.23±0.010def	8.993 ± 0.036 fgh	10.467±0.096 cdef	19.460±0.042 def	145.74±0.014 e
LAYH 15	7.60±0.193 bcd	0.830±0.090 a	151.45±0.012 bcd	8.60±0.112 gh	10.880 ± 0.072 bcdef	19.48±0.053 def	191.09 ±0.014 b
LAYH 16	8.600 ±0.118 bcd	0.7267±0.093 ab	152.63±0.007 bcd	7.530 ±0.122 h	12.123± 0.133 abcd	19.737± 0.126 def	167.47±0.008 d

Different letters in the same column indicate significant mean differences ($p < 0.05$) according to Turkey's test.

C2A and C2B. C2A contained two accessions i.e. LAYH 12 and BHKR6, whereas C2B contained six accessions i.e. LAYH 15, BHKR10, BHKR3, BHKR8, LAYH13 and BHKR9 (Figure 7).

Wild genetic resources are adapted to diverse climatic zones and their adaptive features can be tracked into modern cultivars through conventional breeding or modern molecular techniques. Capability of *Ziziphus* species to cross freely has permitted the buildup of diverse gene pool which owned massive heterozygosity towards climatic adoptability, morphological attributes and genomic DNA contents. Traits evaluated in this study were used previously in characterization of jujubes (Ahmad *et al.*, 2016; Amin *et al.*, 2018) and other fruit crops (Andres-Augustin *et al.*, 2006; Rodriguez *et al.*, 2008; Awasthi and More, 2009).

Somatic mutations are the main source of variability in wild jujube accessions (Geleta *et al.*, 2006). Morphological characterizations are easy and cheap but are usually prone to phenotypic plasticity (Mondini *et al.*, 2009).

Present study quite efficiently scrutinizes variations within accessions and reveals unique traits for breeding and profitable jujube farming. Computed diversity in this study can be enormously applicable towards promoting jujubes selection, breeding and conservation. Morphological diversity showed that accessions like BHKR7, BHKR2, BHKR5 and

LAYH16 had high fruit weight and the accessions BHKR8, BHKR10, BHKR7 had small thorns length. The high fruit weight and small thorns size in inbred lines could be attractive features to consider these accessions as breeding parents. The valuable information about tree, leaf, fruit and stone morphology can be used efficiently to estimate genetic relationships among diverse populations of jujube. It has been demonstrated that fruit weight is remarkable diverse trait in jujube germplasm. Our findings are quite significant and supported by previous findings in China (Liu and Cheng, 1994; Wang *et al*., 1999; Gao *et al*., 2009) India (Tomar and Singh, 1987; Singh *et al*., 2002; Chesfeeda *et al*., 2013; Shiwanand and Bhagwan, 2018), Iran (Tatari *et al*., 2016), Saudi Arabia (Obeed *et al*., 2008), Russia (Akhundova and Agaev, 1989), Bangladesh (Ara *et al*., 2008) and Pakistan (Razi *et al*., 2013; Ahmad *et al*., 2016). Obeed *et al.* (2008) described tremendous diversity in jujube regarding stem diameter, tree height, and canopy breadth.

Table 9: Descriptive statistics of hunter color of selected jujube accessions of Thal desert.

Accessions	Descriptive statistics	L	a*	b*	h°	C
Bhakkar accessions	Average	48.2	2.7	13.0	86.9	13.4
	Maximum	50.4	8.2	18.0	83.8	19.8
	Minimum	38.6	5.1	15.6	78.2	16.4
	Standard deviation	3.4	2.4	3.4	3.9	3.7
	Coefficient variation (%)	7.6	91.0	25.9	4.5	27.5
Layyah accessions	Average	46.7	3.8	16.4	88.7	16.6
	Maximum	49.6	4.2	34.5	89.3	34.7
	Minimum	45.0	3.9	16.2	86.2	16.8
	Standard deviation	1.8	1.6	9.4	1.4	9.4
	Coefficient variation (%)	3.9	74.0	57.0	1.5	56.7

Values presented are mean of triplicate analysis. Chroma (C) = ((a*) 2 + (b*) 2)1/2 and hue angle (h°) = arc tan (b*/a*).

Deviation in fruit characteristics among the selected accessions clearly demonstrated a demarcation in genotype even with similar geo-climatic conditions about the range in fruit weight (1.88 to 4.72g) entailed that this trait can be employed during selection when fruit weight is an intention for domestication/ breeding. The small and large fruit size accessions existed in Layyah and Bhakkar zones; however, accessions with large fruit length, fruit width and large fruits weights are preferred by the consumers. Small stone size/ weight is another important trait for germplasm selection, which in this study deviated from 0.32 to 1.09g. The significant positive correlation and strong relationship between fruit weight and fruit length, fruit length and fruit width, leaf length and stone weight, fruit width and stone weight were established in present study, and such traits can be used for indirect selection. Weak or negative relationships established in some traits manifest that indirect selection may not be practicable in such traits of jujube germplasm.

3.6 Biochemical analysis

Biochemical diversity among investigated wild jujube accessions is shown in Table 8. The highest TSS (11.80%) was governed by BHKR4 followed by BHKR7 (9.20%), BHKR10 (9.10%) and LAYH16 (8.60%), whereas the lowest TSS was governed by LAYH13 (5.9%) followed by BHKR2 (5.83%) and BHKR9 (5.93%). Maximum acidity was noted in LAYH15 (0.830%) and the minimum in BHKR8 (0. 24%).Vitamin C contents were highest (164.69 mg/100g) in BHKR4 followed by BHKR8 (163.56 mg/100g), BHKR10 (156.38 mg/100g) and BHKR1 (155.11 mg/100g).Maximum reducing sugar (15.50%) was recorded in BHKR5 followed by BHKR6 (13.62%) and minimum in LAYH16 (7.53 %). The highest on reducing sugar contents were examined in BHKR8 (13.66 %) and the lowest in BHKR3 (3.15 %). Maximum total sugar contents were yielded by BHKR8 (26.52 %) followed by BHKR4 (25.00 %), BHKR10 (24.99 %) and BHKR5 (23.75 %). Total phenolic contents were high in BHKR5 (247.68 µg GAE mL^{-1}) followed by LAYH13 (246.41 µg GAE mL^{-1}) and the lowest inLAYH12 (131.92 µg GAE mL^{-1}).

Wide range of diversity based on biochemical characterization was documented in selected wild jujube accessions. Biochemical distinctions present significant knowledge for breeding programs (Awasthi and More, 2009). Generally, jujube fruit quality is altered by cultivar specificity environment and agronomic practices (Gao *et al*., 2011; Kumar *et al*., 2012).

Accessions with high range of TSS can be asexually propagated and conserved as novel desi jujube strains whereas high in acidic contents can be used for industrial purposes. Total soluble solid is a critical maturity index and trade mark that is utilized for cultivar cataloging and varietal registration (Ghosh and Mitra, 2004; Tomar and Singh, 1987; Gupta *et al*., 2003). Total soluble solid contents and fruit acidity ranged from 5.9 to 13.2% and 0.29 to 0.82%, respectively in selected wild jujube germplasm. This variation might be due to uniqueness of genotype, environmental influences or genetic constitution of wild ecotypes.

Ketipearachchi *et al*. (2015) noted comparatively low peak of TSS range (1.6 to 16 °Brix) among *Ziziphus* accessions growing in desert zones (Dry Dambulla, Hambantota and Putlam) of Sri Lanka. The accounted range of fruits TSS and acidity was agreed with those investigated in Spanish (Galindo *et al*., 2015; Rechea *et al*., 2018, 2019), Chinese (Gao *et al*., 2011, Gao and Wang, 2012; Wu *et al*., 2012) and Turkish jujube varieties. Broad range TSS diversity has also been documented in Chinese jujube by Preeti and Tripathi (2014), whereas Islam (2007) accounted narrow range for total soluble solid contents among Bangladeshi jujube cultivars i.e. from 12.00 (Apple kul) to 15.00% (Myanmar kul). Vitamin C contents ranged in selected jujube germplasm from 131.12 to 165.56 mg/100g, whereas Anjum *et al*. (2018) discovered low range of vitamin C contents in domesticated Pakistani jujube cultivars (22.22-72.53mg/100ml). However, Pathare *et al*. (2016) mentioned that ascorbic acid contents were high in wild genotypes. Among investigated jujube germplasm reducing sugars, non-reducing sugars and total sugars ranged 7.15 to 16.1%, 2.37 to 13.67 % and 16.54 to 27%, respectively. Pareek *et al*. (2009), Ghosh and Mathew, (2002) and Godi *et al*. (2016) also recorded similar variations for sugar contents among Indian jujube cultivars.

The differences among the cultivars for sugars were possibly due to genetic composition, natural environmental deviation and fruit position on tree in respect to sunlight. This study revealed that accessions with demanding biochemical attributes are significant as breeding parents to evolve new commercial jujube cultivars.

3.9 Color meter analysis for fruit

The data regarding fruit color of selected jujube accessions were subjected to descriptive statistical analysis (Table 9). Average +a* value (indicating red color) recorded for Bhakkar accessions was 2.7, while for Layyah accessions it was 3.8. Average +b* value (depicting yellow portion) was 13.0 for Bhakkar accessions and 16.4 was accounted for Layyah accessions. L*i.e. lightness to darkness of color, was counted with average value 48.2 for accession Bhakkar while for Layyah accessions it was 46.7. Chroma value indicating vividness of color was quantified 13.4 for Bhakkar accessions and 16.6 for Layyah accessions. The values ranged from 38.6 to 50.4 for L*, 5.1 to 8.2 for +a*, 15.6 to 18.0 for +b*, 78.2 to 83.8 for h°, 16.4 to 19.8 for C of Bhakkar germplasm, whereas Layyah gene pool values deviated from 45.0 to 49.6 for L*, 16.2 to 34.5 for +b*, 86.2 to 89.3 for h°, and 16.8 to 34.7 for C.

Jujube fruit color development during maturation is due to changes in the levels of flavonoids, anthocyanins, carotenoids and antioxidant activity. Wild jujube attains yellowish green or chocolate brown color when it ripens and matures physiologically. Consumer preference is strongly influenced by a number of factors including flavor, texture and taste among which fruit color has prime importance (Spinnler *et al*., 1996; Harker *et al*., 2003).

Conclusions and Recommendations

This study provided valuable information about morphological and biochemical characteristics of *Ziziphus nummularia* to identify novel genotypes as well as signify the prevailing diversity in Thal desert. Accordingly, this information could be effective for future breeding programs aimed at developing and producing superior genotypes as well as for designing conservation strategies to prevent loss of this crucial diversity. Findings are also valuable for fruit processing industry to uplift the scope of this neglected crop. Multivariate analysis based on morphological attributes showed strong divergence among investigated gene pool and declared accessions BHKR2, BHKR4 BHKR5, BHKR7 and LAYH16 are superior for germplasm conservation. These genotypes had high values for most of the governed traits and breeders could can select these genotypes for specific breeding purposes. Study also concluded that this neglected *Ziziphus* species of Thal zone is highly considerable to combat malnutrition. Finally, conservation of this auspicious variation is highly recommended to save this rapidly extincting tree species. In future, valorize this study, the number of jujube accessions may be extended to know the genetic diversity in more detail. Diversity estimation by applying molecular markers can further identify agronomically important genes.

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Novelty Statement

Significant variation was detected among investigated jujube accessions for morphological, biochemical and color attributes. Hence, the present study can provide insights for enrichment of existing jujube germplasm of country by exploring the Thal zone of Punjab.

Author's Contribution

Naseem Sharif: Did Experiment and wrote manuscript.
Muhammad Kashif Raza and Urwa Irshad: Supported throughout experiment.
Imran Muhammad Siqqique, Muhammad Ikhlaq Khan and Muhammad Ahsan Qureshi: Helped in assessing biochemical traits and manuscript writing.
Mohsin Abbas and Muhammad Maaz Aziz, Sitwat Riaz and Komal Aslam: Helped in the field data collection and provided help in statistical data analysis.
Naseem Akhtar: Helped in soil analysis and manuscript writing.

Conflict of interest

The authors have declared no conflict of interest.

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