

Genetic diversity in Jujube germplasm (*Ziziphus jujuba* Mill.) based on morphological and pomological traits in Isfahan province, Iran

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ABSTRACT

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Identifying and selecting superior genotypes in native germplasm is one method for breeding fruit trees. Five different ecotypes of Jujube (*Ziziphus jujuba* Mill.) were collected from different regions of Isfahan province, Iran, for evaluation of their morphological and pomological traits during 2011-13. Results showed that quantitative traits were more significant within ecotypes. ‘Najafabad’ ecotype had the highest dimensions of leaves (48×28 mm), fruit weight (2.1 g), and stone weight (0.35g). The largest fruit width (17mm) and peduncle length (13mm) was observed in ‘Ardestan’ ecotype, whereas the largest fruit length (22mm) was observed in ‘Dehaghan’ ecotype. According to the results, the smallest size and weight of fruit, stone weight, and the longest and highest number of annual thorns in shoots were measured in ‘Kouhpayeh’ ecotype. Results showed significant negative and positive correlations between some traits. According to the cluster analysis, ecotypes with desirable traits of fruit were placed in separate clusters from other ecotypes. ‘Najafabad’ ecotype, followed by ‘Ardestan’ and ‘Dehaghan’ ecotypes, can be recommended as promising ecotypes for establishing Jujube orchards and use in Jujube breeding programs in Iran.

Key words: cluster analysis, ecotype, genetic variation, Jujube, morphological traits,

INTRODUCTION

Jujube (*Ziziphus jujuba* Mill.) belongs to the “Rhamnaceae” family and produces a tree about 6-8 m high. The *Ziziphus* genus has about 45 diploid species, each with 24 chromosomes (Liu, 2003; Mukhtar *et al.*, 2004). The species is self-incompatible and requires pollinizer genotypes to obtain maximum yields (Radicevic *et al.*, 2013). Jujube was first cultivated 7700 years ago in China and was transmitted via the Silk Road to other parts of the world such as India, Iran, Afghanistan, and Central Asia. While some species have temperate to sub-tropical origins, Jujube is naturally adapted to areas with cold winters, hot summers, and dry climates.

Jujube is tolerant to some environmental stresses such as water deficit and salinity, as well as some pests and diseases. Its fruits are organic, because it is produced with less pesticides (Velkoska-Markovska and Petanovska-Ilievska, 2013). Easy harvesting, high yield and price, wide range of adaptability, easy management, early bearing, and food enrichment are unique features of jujube.

Flowering and fruit set in jujube occur in several

continuous steps. The first flowering is in April and fruits need about 100 days to mature. The second flowering occurs in June; these fruits are medium-sized and need 60-75 days to mature. The third flowers open in July and produce small fruits (called Mehrgoun, in Iran; Kohandel *et al.*, 2011) that need 45-50 days to mature. Jujube fruit is sweet and rich in vitamin A, B, C as well as minerals and different components of alkaloid, flavonoid, sterol, tannin, saponin, and fatty acids (Zhao *et al.*, 2006). Jujube also has significant amounts of antioxidants that can neutralize the activity of free radicals (Li *et al.*, 2005), thus the fruit has a role in traditional medicine.

Some traits have been proposed as important for classifying Jujube cultivars. Liu *et al.* (2013) investigated Jujube cultivars in Gravel Gobi, Southern Xinjiang, China. They introduced some suitable varieties for these areas according to fruit characteristics, and proposed their results as a reliable reference for classifying Jujube varieties in Xinjiang. Significant differences in morphological variation of 29 clones of Jujube in the Loess Plateau were also found by Gao *et al.* (2009), who observed

significant diversity between leaf length and width of Jujube genotypes. Similarly, Obeed *et al.* (2008) reported large diversity in tree height, trunk diameter, and canopy width in Jujube, and proposed that these traits are the most reliable characteristics for classifying Jujube varieties. However, Bal (1992) prepared an identification key for different cultivars of *Z. mauritiana*, which is from the Jujube family, and concluded that the shape of leaf tip is the most reliable characteristic for classifying cultivars.

Gao *et al.* (2003) reported that Jujube fruit weight varied from 15.3 g in 'Zanhuangzao' to 25.7 g in 'Lizao' varieties. Ghazaian (2015) studied 10 ecotypes of Jujube collected from different areas of Golestan province, Iran and concluded that climatic conditions significantly affected both quantitative and qualitative traits of Jujube fruits. In that study, ecotypes 'Charmzoo' and 'Takhshi' had the highest (2.2 cm) and lowest (1.06 cm) thorn length, respectively (Ghazaian, 2015), while Khakdaman *et al.* (2007) reported 2-5 cm variation in thorn length, and a significant correlation with leaf length. Liu *et al.* (2009), showed that mean fruit weight of Jujube varieties ranged from 0.14-6.33 g. Sivakov *et al.* (1988) reported that the mean stone weight of six Jujube varieties varied from 0.28-0.65 g, while Ghosh and Mathew (2002) reported stone weight variation of 0.06-1.9 g.

Studying the genetic diversity of any plant species provides important information for breeding programs (Soltani *et al.*, 2011) as natural populations are a useful source of diversity (Awasthi and More, 2009). Identifying local genotypes with desirable fruit characteristics, adaptability, and tolerance to biotic and abiotic stresses is therefore essential. Shahhoseini *et al.* (2012) proposed that the central regions of Iran are one of the origins of Jujube, and it has been grown in some provinces of Iran, such as Isfahan (Ghous *et al.*, 2014).

Cropping pattern in Isfahan province have changed due to increasing temperatures and water shortages, thus highlighting the need to identify jujube ecotypes with less chilling requirements and tolerance to drought and heat stresses. As such, this study aimed to identify diversity within the Jujube germplasm of Isfahan province, Iran, and classify ecotypes according to vegetative and reproductive traits, in order to recommend them for orchard establishment or Jujube breeding programs.

MATERIALS AND METHODS

Plant materials

Five Jujube ecotypes were collected from

different areas of Isfahan province and evaluated during 2011-13 (Fig. 1). Vegetative and reproductive traits were measured and recorded for Jujube ecotypes in four stages: 1) physiological dormancy; 2) flowering; 3) active vegetative growth; and 4) ripening and harvesting. Ecotypes from each region were labeled and their geographical information (longitude, latitude, and altitude) was recorded using a GPS device (Table 1). A map of Isfahan province and the collection sites is shown in Fig. 1.



Fig. 1. Map of Isfahan Province and collection sites of Jujube ecotypes.

Table 1. Geographical coordinates of collection sites of Jujube ecotypes in Isfahan Province

Collection site	Latitude	Longitude	Altitude (m)
Najafabad	32 28' 20.04" N	51 21' 49.00" E	1643
Ardestan	33 17' 11.06" N	52 28' 39.08" E	1622
Dehaghan	32 07' 54.07" N	51 38' 57.05" E	1822
Kouhpayeh-1	32 42' 31.06" N	52 26' 13.05" E	1764
Kouhpayeh-2	32 42' 31.069" N	52 26' 13.05" E	1764

Morphological and pomological traits

To evaluate each Jujube ecotype, 26 vegetative and reproductive traits (including different morphological and pomological traits) were measured/recorded following the International Plant Genetic Resources Institute (IPGRI) descriptor (Saha, 1997). Traits, abbreviations, and measurement methods are given in Table 2. One representative plant from each ecotype was selected and its traits were measured/recorded. Some suckers were taken from five selected ecotypes and sent to the National Collection of Jujube in Birjand, Iran.

Data analysis

Analysis of variance and comparison of means for quantitative traits were performed using SAS (version 9.1). Descriptive statistics, simple correlations between traits (Pearson method), and cluster analysis and grouping of ecotypes (Ward method and on the basis of squared

Table 2. Abbreviation and measurement methods for morphological and pomological traits.

Trait	Abb.	Unit	Measurement method
Tree vigor	TV	Code	Extremely weak (1), Weak (2), Intermediate (5), Vigorous (7), Extremely vigorous (9)
Tree growth habit	TGH	Code	Upright (1), Semi upright (3), Semi spreading (5), Spreading (7), Drooping (9)
Canopy width	CW	m	Tape meter
Leaf length	LL	mm	Ruler
Leaf width	LW	mm	Ruler
Shape of leaf tip	SLT	Code	Globular (1), Taper (3), Sharp (5)
Shape of leaf margin	SLM	Code	Smooth (1), Fine serrated (3), Serrated and lobed (5)
Leaf adaxial color	LDC	Code	Light green (1), Green (3), Dark green (5), Very dark green (7)
Leaf abaxial color	LBC	Code	Light green (1), Green (3), Dark green (5), Very dark green (7)
Annual shoot length	ASL	cm	Ruler
Number of thorns in shoot	NTS	Number	Enumeration
Annual thorn length	ATL	Cm	Ruler
Flowering time	FT	Code	Very early (1), Early (2), Almost Early (3), Middle (4), Almost late (5), Late (6), Very late (7)
Flowering habit	FH	Code	Annual shoot (1), Spur (3), Annual shoot and spur (5)
Number of flowers per cyme	NFC	Number	Enumeration
Ripening time	RT	Code	Very early (1), early (2), Almost early (3), Middle (4), Almost late (5), Late (6), Very late (7)
Fruit shape	FS	Code	Globular (1), Small cylindrical (3), Cylindrical (5), Long cylindrical (7)
Fruit length	FL	mm	Caliper
Fruit width	FWI	mm	Caliper
Fruit weight	FW	g	Digital scale
Fruit cover	FC	Code	Downy (1), Glabrous (3)
Peduncle length	PL	mm	Ruler
Stone size	SS	Code	Small (1), Medium (3), Large (5)
Stone weight	SW	g	Digital scale
Stone surface	SSU	Code	Smooth (1), serrated (3)
Flesh/stone ratio	FSR	Code	Low (1), Medium (3), High (5)

Euclidean distance) was carried out using SPSS (version 15).

RESULTS

Results of descriptive statistics for each trait are shown in Table 3. Coefficient of variation varied

from 0 to 35.83%; traits with high coefficient of variation have a wider range and provide more variation for that trait. Traits such as number of thorns, annual thorn length, fruit weight, shape and width, as well as stone weight and size had coefficient of variation more than 30%.

Table 3. Mean, standard deviation (Stdev.), range and coefficient of variation (CV) for studied traits.

Characteristics	CV (%)	Stdev. (±)	Minimum	Mean	Maximum
CW	25.90	1.14	3.0	4.4	6.0
LL	5.29	2.54	44.0	48.0	51.0
LW	18.6	4.39	18	23.6	28.0
ASL	13.82	4.81	27.0	34.8	40.0
NTS	35.83	2.58	5.0	7.2	10.0
ATL	33.33	1.18	0.3	1.44	3.1
NFC	4.48	0.44	9.0	9.8	10.0
FL	27.43	4.50	10.0	16.4	22.0
FWI	31.81	4.2	6.0	13.2	17.0
FW	35.64	10.26	18.4	28.78	42.0
PL	27.88	6.5	0.6	5.38	13.0
SW	31.97	1.72	3.1	5.38	7.0
TV	29.59	2.19	5.0	7.4	9.0
TGH	0.00	0.00	1.0	1.0	1.0
SLT	25.95	1.09	3.0	4.2	5.0
SLM	0.00	0.00	3.0	3.0	3.0
LDC	0.00	0.00	3.0	3.0	3.0
LBC	0.00	0.00	1.0	1.0	1.0
FT	19.76	0.83	3.0	4.2	5.0
FH	0.00	0.00	5.0	5.0	5.0
RT	16.48	0.89	4.0	5.4	6.0
FS	35.23	1.78	3.0	4.2	7.0
FC	0.00	0.00	3.0	3.0	3.0
SS	34.23	0.89	1.0	2.6	3.0
SSU	0.00	0.00	1.0	1.0	1.0
FSR	28.68	1.09	3.0	3.8	5.0

For more details of abbreviation and units for different traits, please see Table 2.

Analysis of variance showed that the ecotypes had significant differences for morphological and pomological traits, which demonstrates diversity

(Tables 4 and 5). The largest and smallest canopy width belonged to 'Kouhpayeh-1' (6m) and 'Dehaghan' (3m) ecotypes, respectively (Table 4).

Table 4. Mean of quantitative traits for five ecotypes of jujube.

Ecotype	CW	LL	LW	ASL	NTS	ATL	NFC	FL	FWI	FW	PL	SW
'Najafabad'	5.00a	48a	28a	27b	6.5b	3.1a	10.66a	18ab	16a	2.1a	6b	0.35a
'Ardestan'	5.00a	51a	21bc	37a	7.5b	1.6b	12.66a	19ab	17a	1.4bc	13a	0.35a
'Dehaghan'	3.00b	44b	28a	35ab	6.0b	1.9b	11.0a	22a	15a	1.8ab	12a	0.28ab
'Kouhpayeh-1'	6.00a	49a	23b	40a	13.66a	0.3c	12.16a	15c	14ab	0.92d	6b	0.15c
'Kouhpayeh-2'	5.00a	48a	18c	35ab	12.66a	0.3c	11.0a	16bc	14ab	0.96dc	7b	0.21bc

-Means, in each column, followed by similar letter (s) are not significantly different at the 5% probability level-using Duncan's Multiple Range Test.

- For more details of abbreviation and units for different traits, please see Table 2.

Table 5. Mean of qualitative traits for five ecotypes of jujube.

Ecotype	TV	TGH	SLT	SLM	LDC	LBC	FT	FH	RT	FS	FC	SS	SSU	FSR
'Najafabad'	5	1	3	3	3	1	3	5	5	3	3	3	1	3
'Ardestan'	5	1	3	3	3	1	5	5	6	5	3	3	1	5
'Dehaghan'	9	1	5	3	3	1	5	5	6	7	3	3	1	5
'Kouhpayeh-1'	9	1	5	3	3	1	4	5	4	3	3	1	1	3
'Kouhpayeh-2'	9	1	5	3	3	1	4	5	6	3	3	3	1	3

-For more details of abbreviation and units for different traits, please see Table 2.

'Najafabad' and 'Dehaghan' ecotypes had the largest (28 mm) and 'Kouhpayeh-2' the smallest (18 mm) leaf width (Table 4). Leaf length varied from 44 mm ('Dehaghan') to 51 mm ('Ardestan'). The longest and shortest annual shoot length were 40 cm ('Kouhpayeh-1') and 27 cm ('Najafabad'), respectively. Number of thorns per shoot ranged from 13.66 ('Kouhpayeh-1') to 6.00 ('Dehaghan') (Table 4). 'Najafabad' ecotype had the greatest annual thorn length (3.1 cm) whereas 'Kouhpayeh-1' and 'Kouhpayeh-2' had the least (0.3 cm). Jujube ecotypes did not significantly differ for number of flower per cyme, though 'Ardestan' had more flowers per cyme (12.66) than other ecotypes (Table 4). The greatest fruit length and width was found in 'Dehaghan' (22 mm) and 'Ardestan' (17 mm) ecotypes, respectively, while the least belonged Kouhpayeh ecotypes (14 and 15 mm) (Table 4).

Fruit and stone weight varied from 0.92-2.1 g and 0.15-0.35g, respectively (Table 4). The greatest fruit and stone weight was found in 'Najafabad', which was 2.3 fold heavier than 'Kouhpayeh-1'. 'Ardestan' ecotype had the longest peduncle (13 mm), but this was not significantly different from 'Dehaghan' (Table 4).

'Najafabad' and 'Ardestan' ecotypes had trees with intermediate vigor while 'Dehaghan' and Kouhpayeh ecotypes were extremely vigorous (Table 5). The shape of leaf tip of 'Najafabad' and 'Ardestan' ecotypes was tapered, compared to sharp leaf tips for the other ecotypes (Table 5).

Flowering time in 'Najafabad' ecotype was almost early, whereas for 'Ardestan' and 'Dehaghan' ecotypes it was almost late, and Kouhpayeh ecotypes had moderate flowering times (Table 5). Ripening time for 'Najafabad' ecotype was almost late, for 'Ardestan', 'Dehaghan', and 'Kouhpayeh-2' ecotypes it was late, while 'Kouhpayeh-1' was medium. 'Najafabad' and Kouhpayeh ecotypes had small cylindrical fruits,

compared to cylindrical for 'Ardestan' and long cylindrical for 'Dehaghan' (Table 5). Stone size in 'Kouhpayeh-1' was small and in the remaining ecotypes it was medium (Table 5). A higher flesh : stone ratio was observed in 'Dehaghan' and 'Ardestan' ecotypes. There was no significant difference among some qualitative traits including tree growth habit (upright), shape of leaf margin (fine serrated), leaf adaxial color (green), leaf abaxial color (light green), flowering habit (annual shoot and spur), fruit cover (glabrous), and stone surface (smooth) (Table 5).

There were some significant positive and negative correlations between some traits (Table 6). For example, number of thorns was negatively correlated with thorn length ($r = -0.918^*$), fruit weight ($r = -0.932^*$), and stone weight ($r = -0.878^*$), but positively correlated with canopy width ($r = 0.898^*$). Flowering time showed a positive correlation with fruit length ($r = 0.968^{**}$) and fruit width ($r = 0.909^*$). Fruit shape was positively correlated with flesh: stone ratio ($r = 0.919^*$) and number of flowers per cyme had positive correlations with fruit width ($r = 0.957$) (Table 6).

Cluster analysis classified ecotypes into three groups at five squared Euclidean distance using the Ward method (Fig. 2). 'Najafabad' ecotype was separate in one group and had the highest fruit weight and thorn length. 'Kouhpayeh-1' and 'Kouhpayeh-2' formed another group, which indicates that they may be same genotype, but different from the other ecotypes. These ecotypes had the most number of thorns in shoot and largest canopy width, as well as the least annual thorn length, fruit length and weight, and stone weight. 'Dehaghan' and 'Ardestan' ecotypes were also located in the same group. This group had the highest fruit and peduncle length.

Table 6. Correlation coefficient for relationship between some traits of Jujube ecotypes.

Traits	CW	LL	LW	ASL	NTS	ATL	NFC	FL	FWI	FW	PL	SW	TV	SLT	FT	RT	FS	SS	FSR	
CW	1																			
LL	0.516	1																		
LW	-0.559	-0.580	1																	
ASL	0.473	0.224	-0.513	1																
NTS	0.898*	0.303	-0.739	0.586	1															
ATL	-0.720	-0.207	0.769	-0.819	-0.918*	1														
NFC	0.196	0.00	-0.560	0.905*	0.475	-0.785	1													
FL	-0.428	-0.348	-0.091	0.569	-0.137	-0.262	0.794	1												
FWI	0.031	0.117	-0.522	0.854	0.271	-0.620	0.957*	0.852	1											
FW	-0.796	-0.397	0.833*	-0.805	-0.932*	0.980**	-0.720	-0.151	-0.584	1										
PL	-0.703	-0.127	0.152	0.236	-0.589	0.231	0.411	0.819	0.618	0.268	1									
SW	0.758	0.097	0.391	-0.636	-0.878*	0.858	-0.525	-0.41	-0.272	0.769	0.501	1								
TV	0.320	-0.537	-0.187	0.531	0.600	-0.703	0.612	0.385	0.369	-0.553	-0.199	-0.858	1							
SLT	0.320	-0.537	-0.187	0.531	0.600	-0.703	0.612	0.385	0.369	-0.553	-0.199	-0.858	0.999**	1						
FT	-0.367	-0.117	-0.177	0.633	-0.139	-0.263	0.802	0.968**	0.909*	-0.197	0.873	0.038	0.218	0.218	1					
RT	-0.686	-0.219	-0.204	-0.151	-0.367	0.147	0.250	0.571	0.372	0.205	0.616	0.477	-0.102	-0.102	0.218	1				
FS	-0.784	-0.548	0.394	-0.151	-0.605	0.255	0.375	0.856	0.492	0.372	0.896*	0.334	0.102	0.102	0.218	0.218	1			
SS	-0.784	-0.219	0.076	-0.604	-0.605	0.539	-0.250	0.174	-0.106	0.565	0.411	0.739	-0.408	-0.408	0.218	0.218	0.218	1		
FSR	-0.721	-0.179	0.187	0.227	-0.600	0.239	0.408	0.831	0.608	0.286	0.999**	0.487	-0.167	-0.167	0.218	0.218	0.218	0.218	1	

*and** = significant at the 1% and 5% of probability levels, respectively.

For more details of abbreviation and units for different traits, please see Table 2.

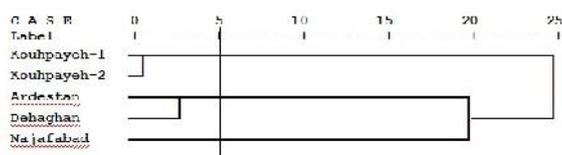


Fig. 2. Grouping of five ecotypes of Jujube based on measured traits using Ward method.

DISCUSSION

The coefficient of variation for different morphological and pomological traits varied from 0 to 35.83% and was greater than 30% for some traits (Table 3). Khakdaman *et al.* (2007) reported that coefficient of variation for petiole length was 3.7% and for other traits varied from 10% to 25%, which shows lower variability in traits in their study. Greater diversity gives more opportunities for selecting new varieties of Jujube (Liu, 2006). Saran (2005) reported high coefficient of variation for stone size, flesh :stone ratio, and fruit weight. Similarly, in this study, higher coefficient of variation was observed for stone size (34.23%), flesh: stone ratio (28.68%), and fruit weight (35.64%) (Table 4). These results concur with Gao *et al.* (2009), who also observed high variation in fruit weight between Jujube clones. Therefore fruit weight is an appropriate trait for classifying Jujube ecotypes.

Leaf length and width varied among the Jujube ecotypes from Isfahan. Ghazaian (2015) reported leaf width variation of 13-24 mm in Jujube ecotypes from Golestan province in Iran. In a similar study, leaf length and width ranged from 19-30 mm and 9-17.5 mm, respectively (Khakdaman *et al.*, 2007), thus the variation in Jujube leaf length and width was greater in this study than that of the ecotypes from Golestan province.

Annual shoot length varied from 27 to 40 cm, compared to 11-30 cm in other studies (Khakdaman *et al.*, 2007; Ghazaian 2015). 'Najafabad' had the longest annual thorn (3.1 cm), while Kouhpayeh ecotypes had the shortest annual thorn (0.3 cm). Khakdaman *et al.* (2007) reported that climatic conditions influence annual thorn length.

The fruit length of ecotypes in the current study (22 mm) was almost the same as previously reported (21.6 mm) by Ghazaian (2015). The largest fruit width in this study corresponded to 'Ardestan' (17 mm), which was lower than the greatest fruit width reported by Ghazaian (2015) for 'Golestan' ecotype (21.3 mm). While fruit length is an important trait, there are not many Jujube varieties with large fruits (Grygorieva *et al.*, 2014).

Fruit weight is one of the most important traits

for breeders (Gao *et al.*, 2003). In this study, fruit and stone weight means varied from 0.92-2.1g and from 0.15-0.35g, respectively. The highest fruit (2.1 g) and stone (0.35 g) weight belonged to 'Najafabad' ecotype. Ghazaian (2015) showed a wider range of fruit weight (0.79-4.8 g) for Jujube ecotypes from Golestan province. Variation in fruit weight depends on the variety and ecological conditions. Varieties with small fruits are suitable for nut production (Gao *et al.*, 2003).

Fruit length varied from 3.27 to 4.33 cm in a study by Kundi and Wazir (1980), and was higher than the fruit length of Jujube ecotypes in this study. Flesh: stone ratio in 'Dehaghan' and 'Ardestan' ecotypes was more than other ecotypes. Differences inflesh: stone ration in Jujube varieties has been previously reported (Kundi and Wazir, 1980; Ghazaian, 2015).

Jujube ecotypes from Isfahan province are an important component of Iran's Jujube germplasm. Clustering analysis showed that 'Kouhpayeh-1' and 'Kouhpayeh-2' were similar and formed one group. These ecotypes had more vegetative growth than other ecotypes. 'Dehaghan' and 'Ardestan' ecotypes were also grouped together, despite being collected from different climatic conditions and locations. Ecotypes belonging to different regions were classified in the same group due to their common origin (Khakdaman *et al.*, 2007).

Ghous *et al.* (2014) reported that Jujube in humid and cold regions has fewer shoot thorns, thinner and smaller thorns, smaller shoots, and taller trees with wider canopies, while Jujube in arid and semi-arid regions has more shoot thorns, thicker and higher thorns, longer shoots, and shorter trees with a more closed canopy. Climatic conditions have large effects on the morphological traits of Jujube ecotypes and their vegetative characteristics. Isfahan province is experiencing climate change; reduced rainfall, drought, and warmer temperatures.

CONCLUSION

Diversity allows for selection of superior ecotypes that have adapted in different regions over many years, as well as identifying ecotypes to preserve them. Understanding the morphological and pomological characteristics of Jujube, as well as quantitative and qualitative traits of fruits, provides opportunities for selection and improvement of the ecotypes cultivated. These results showed that 'Najafabad', 'Ardestan', and 'Dehaghan' ecotypes are suitable for cultivation in Isfahan Province for establishing commercial Jujube orchards.

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