e-ISSN: 2177-5133
www.ufpi.br/comunicata

Comunicata Scientiae 6(1): 10-16, 2015

Original Article

Genetic diversity of Jujube (*Ziziphus jujuba* Mill.) germplasm based on vegetative and fruits physicochemical characteristics from Golestan province of Iran

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Abstract

Jujube fruits have sweet flavor and are rich of A, C and B vitamins and mineral nutrients. It used as table fruit, nut fruit, medicinal and ornamental plant. In this study 10 different ecotypes of *Ziziphus jujuba* Mill. were collected from different regions of Golestan province of Iran and their vegetative (stem annual growth, thorn length, leaf length, leaf width and petiole length) and fruit physicochemical characteristics (fruit length, fruit width, fruit weight, humidity percent, flesh/stone ratio, stone weight, stone length, stone width, vitamin C content, pH and total soluble solid (TSS)) were evaluated between years 2009-2011. The results of this study showed significantly differences between ecotypes from different regions. Different evaluated traits of Lemesk ecotype were significantly different from the other ecotypes; this result was corroborated by cluster analysis. The ecotypes were separated according to their geographic origin. So it can be concluded that the climate effects is very important for existence of qualitative and quantitative characteristics of jujube.

Keywords: genetic variation, morphological traits, *ziziphus* ecotypes evaluation, *ziziphus* breeding.

Diversidade genética de Jujuba (*Ziziphus jujuba* Mill.) baseada em germoplasma de frutos com características físico-químicas da província de Golestan do Irã

Resumo

As frutas de Jujuba tem sabor doce e são ricos em vitaminas A, C e do complexo B, nutrientes e minerais. É usado como fruta de mesa, para fins medicinais e como planta ornamental. Neste estudo 10 ecótipos diferentes de *Ziziphus jujuba* Mill. foram coletadas de diferentes regiões da província de Golestan do Irã e sua porção vegetativa (caule de crescimento anual, comprimento espinho, folha de comprimento, largura e comprimento do pecíolo da folha) e as características físico-químicas de frutas (comprimento e largura de fruto, peso do fruto, por cento de umidade, carne / pedra ratio, peso de pedra, pedra comprimento, largura pedra, teor de vitamina C, pH e sólidos solúveis totais (SST)) foram avaliadas entre os anos de 2009-2011. Os resultados deste estudo mostraram diferenças significativas entre ecótipo a partir de diferentes regiões. Diferentes características avaliadas de Lemesk ecotype foram significativamente diferente dos outros ecótipos; esse resultado foi corroborada pela análise de cluster. Os ecótipos foram separados de acordo com sua origem geográfica. Assim, pode-se concluir que os efeitos do clima é muito importante para a existência de características qualitativas e quantitativas de jujuba.

Palavras-chave: variação genética, características morfológicas, avaliação ecótipos Ziziphus, cultura de ziziphus.

Received: 10 October 2013
Accepted: 06 March 2014

Introduction

Jujube (*Zizyphus jujuba* Mill.) is belong to the family Rhamnaceae, is native to Asia and Caucasus, a deciduous tree with 6-8 and sometimes 12 meters height, has resistance to environmental stresses such as water deficit, chilling, salinity, high temperature, pest and diseases. Easy harvesting, high yield, high price are especial features of jujube. Also, it is becoming increasingly popular for its wide adaptation, easy management, early bearing, rich nutrition, and multi-use. The jujube tree has great commercial importance owing to the usefulness of almost all its parts. Jujube fruits can be used either as dried fruits or as fresh fruits. In addition, jujube fruits are used as a medicinal plant in traditional medicine in Iran. The genus *Zizyphus* has about 45 species that are all diploid with 24 chromosomes (Jawanda & Bal, 1978; Liu, 2003; Meena et al., 2003; Mukhtar et al., 2004). Flowering and fruit set in jujube are in several continuous steps. The first generation fruits that are produced from April flowers are large fruits and they need about 100 days to maturation (from bloom to mature). The second generation fruits that are produced from June flowers have medium size and need about 60-75 days to be mature. The third generation fruits are produced from July flowers, they are small fruits that need for 45-50 days to be mature. In Iran the third generation fruits are called as 'Mehrgoun' jujube (Kohandel et al., 2011).

Jujube fruits have sweet flavor and are rich of A, C and B vitamins and mineral nutrients. It also is rich of different alkaloid compounds, flavonoids, sterol, tannins, saponin and fatty acids (Croueour et al., 2002; Abdel-Zaher et al., 2005; Bhargava et al., 2005; Zhao et al., 2006). Also, jujube has valuable amount of antioxidants that are able to neuter of free radical's activity (Li et al., 2005).

As stated earlier genetic variability within a species is the basis for any systematic tree improvement program. Improvement programs should start by determining the important traits and the improvement carried out through the use of variation in natural populations (Awasthi & More, 2009). In each country, study of genetic diversity of a plant species is so important in order to use it in breeding, conservation, management, revival, establishing survive plant covers and new high yielding orchards programs (Soltani et al., 2011). Thus, identification of local genotypes is important to recognize genotypes with good fruit properties, environment adaptation and resistant to biotic and abiotic stresses. The morphological diversity of 29 *Ziziphus jujuba* Mill. clones from Losses Plateau of China were studied by Gao et al. (2009). According to their study, significant differences were found among morphological variations of different traits. Also, Liu et al. (2009a) evaluated sour jujube (*Ziziphus acidojujuba* C.Y. Cheng et M.J. Liu) germplasm in China according to agronomic diversity. Their results showed a very high agronomic diversity among sour jujube accessions. In another study, Liu et al. (2009b) studied properties of fruiting and seed development in Chinese jujube (*Ziziphus jujuba* Mill.). Based on their results, there were huge differences among characteristics of flowering, fruiting and seed development.

In a study, Dong et al. (2009) evaluated distribution of biological characters in *Ziziphus jujuba* Mill. germplasm existed in a Chines pomology institute. They found that Chinese jujube has high differences of variation coefficient among biological characters. They suggested that their findings will have useful information for use of available Chinese jujube cultivars for future research and commercial purposes. Liu et al. (2013) studied table cultivars of Chinese jujube (*Ziziphus jujuba* Mill.) in Gravel Gobi of Southern Xinjiang. They introduced some proper cultivars for this area according to different fruit characteristics. They suggested that their results could provide a reliable reference for readjustment of jujube cultivars in Xinjiang.

Morphological variation is a foundation of plant evolution and new cultivar formation. The aims of this study were to identify genetic diversity of jujube germplasm existed in Golestan province of Iran and classify them according to their vegetative and fruit physicochemical characters.

Materials and Methods

This research was carried out during 2009-2011. Ten different ecotypes were identified and collected from different regions of Golestan

province of Iran (Table 1). Golestan province is between 36.24-38.05°N longitude and 53.51-56.04°E latitude.

Table 1. List of 10 *Ziziphus jujuba* Mill. ecotypes used in this study with their origin and collection place altitudes

Altitude (meter)	City	Location (ecotype)
306	Kalale	Aidarvish
262	Kalale	Charmazoo
60	Gonbad	Igder
107	Gonbad	Gedaige
150	Ramian	Sefid Cheshme
374	Ramian	Seid Kalate
64	Gorgan	Masoum Abad
78	Gorgan	Takhshi Mahale
89	Gorgan	Roshan Abad
32	Gorgan	Lemesk

According to the jujube descriptor, different vegetative (stem annual growth, thorn length, leaf length, leaf width and petiole length) and fruit (fruit length, fruit width, fruit weight, humidity percent, flesh/stone ratio, stone weight, stone length, stone width, vitamin C content, pH and total soluble solid (TSS)) characteristics were evaluated. Stem annual growth, thorn length, leaf characters and petiole length were measured by a ruler. Fruit length, stone length and stone width were measured by a caliper. All characters related to the weight were measured by a digital balance (0.001 g sensitivity). For calculating humidity percent, after measuring fruits fresh weight they transferred to an oven with 60 °C temperature for 24 h and then fruits dry weight were measured then humidity percent was calculated using below formula:

Humidity percent= (fruit fresh weight – fruit dry weight) × 100

TSS was measured by using a refractometer (ATAGO-ATC-20E, Japan). The method of titration with dichlorophenolindophenol was used for measuring vitamin C content (AOAC, 1984) and pH was measured by using a ph meter.

All plants were collected from their natural origin and there were no treatments on them. For measuring characters we used 20 fruit and leaf samples. The survey was carried out according to the *Ziziphus* descriptor.

Data analysis was done using SAS software and means comparison were done using LSD test at 5% level of probability. Cluster analysis was carried out using SPSS® ver. 11.0 for Windows. A dendrogram of genetic similarities among genotypes was compiled using the Ward method.

Results and Discussion

The analysis of variance showed that all parameters were significant. Mean comparison of quantitative and qualitative parameters for each ecotype are shown in Table 2 and 3. Means comparison showed significant differences among studied parameters. Vitamin C content and pH of fruits in Lemesk ecotype were the highest in comparison with other ecotypes and the highest amount of TSS was found in Seid Kalate ecotype. Fruit lengths of Roshan Abad and Gedaige ecotypes were high in comparison with the others. Masoum Abad and Roshan Abad produced the widest fruits in comparing to the others. Gao et al. (2009) found great variation in fruit weight among the clones of *Ziziphus jujuba* Mill..

Regarding to vegetative traits, Lemesk and Aidarvish ecotypes had the highest (5.6 cm) and the lowest (2.5 cm) leaf length, respectively. The highest and the lowest leaf width were shown in Roshan Abad (2.46 cm) and Gedaige (1.7 cm) ecotypes, respectively. Gao et al. (2009) showed significant variations among leaf length and width of *Ziziphus jujuba* Mill. genotypes in the Losses Plateau of China. The highest and the lowest stem annual growth were recorded in Lemesk (30.7 cm) and Aidarvish (11.46 cm) ecotypes, respectively. The highest thorn length was showed at Charmazoo (2.2 cm) ecotype and the lowest one was showed at Takhshi Mahale (1.06 cm) ecotype.

The longest and the lowest petiole lengths were found in Lemesk (3.9 mm) and Igder (0.2 mm) ecotypes, respectively.

Regarding fruit and stone weight, Gedaige (4.8 and 0.29 g, respectively) and Takhshi Mahale (2.11 and 1.93 g, respectively) ecotypes had significant differences in comparison with the other ecotypes. Also, Aidarvish had the highest stone length and stone width in comparison with the others. Liu et al. (2009a) showed that average fruit weight among germplasms ranged from 0.14 to 6.33 g.

The highest ratio of fruit flesh/stone were showed in Gedaige (15.5) and Roshan Abad (15.54) ecotypes and the lowest was found in Takhshi Mahale (0.09) ecotype.

In this research, vitamin C content (mg in 100 g fruit flesh) varied from 40.33 (Takhshi Mahale ecotype) to 284.2 (Lemesk ecotype) within evaluated ecotypes. In other studies, vitamin C contents were reported between 180.11 to 376.3 mg/100 g fruit flesh (Sivakov et al., 1988; Ecevit et al., 2008). Average vitamin C content is 13 mg in 100 g dry fruit and 69 mg in 100 g fresh fruit (Sivakov et al., 1988; Ecevit et al., 2008).

TSS amounts were varied between 16.33 (Golestan ecotypes) to 34.1 (Seid Kalate ecotype). According to the other reports, TSS amounts can be varied from 15 to 45 in *Ziziphus jujuba* Mill. genotypes (Ma et al., 2000; Ghosh et al., 2002; Gao et al., 2003; Chen et al., 2006; Jiang et al., 2006).

Stone weights were different from 0.26 (Lemesk ecotype) to 1.93 (Takhshi Mahale ecotype). Sivakov et al. (1988) reported that the means of stone weight in six *Ziziphus jujuba* Mill. cultivars were varied from 0.28 to 0.65.

Table 2. Means comparison of fruit traits in *Ziziphus jujuba* Mill. ecotypes

Ecotype	pH	TSS	Vitamin C (mg/100g fruit flesh)	Humidity %	Flesh/ stone	Stone-weight (g)	Stone-width (cm)	Stone-length (cm)	Fruit-weight (g)	Fruit-width (cm)	Fruit-length (cm)
Aidarvish	4.6e*	24.46bc	265.66ab	96.93a	7.53bc	0.37b	1.35a	0.79a	3.1bc	1.7c	1.63c
Charmazoo	5cd	18.9de	235.8abc	98.36a	1.14d	0.36b	1.18abc	0.59c	0.79f	1.5d	1.53c
Igder	4.8ed	16.33e	147.8cd	98.46a	5.03cd	0.29b	1.23abc	0.78a	1.78e	1.46d	1.53c
Gedaige	5.2abc	29.03b	176.8bcd	72.76d	15.5a	0.29b	1.31ab	0.75ab	4.8a	2.03ab	2.16a
Sefid Cheshme	4.6e	16.36e	190.53bcd	81.36c	5.51cd	0.47b	1.13abc	0.66abc	2.63d	1.96b	1.93b
Seid Kalate	5.3ab	34.10a	149.13cd	76cd	6.23bcd	0.27b	1.22abc	0.74ab	1.94e	2.06ab	2ab
Masoum Abad	5.1cd	22.1cd	130.16d	73.7d	9.37abc	0.27b	1.13abc	0.69abc	2.74cd	2.13a	2.1ab
Takhshi Mahale	5bcd	20.56cd	40.33e	89.3b	0.09d	1.93a	1.13abc	0.68abc	2.11e	1.56d	1.56c
Roshan Abad	5cd	21.06cde	107.03de	74.86d	15.54a	0.4b	1.06bc	0.61bc	3.52b	2.13a	2.13a
Lemesk	5.4a	25.4bc	284.2a	87.6b	11.92ab	0.26b	1.02c	0.38d	2.94cd	1.53d	1.56c

*Means with the same letter are not significantly different using LSD test.

In Ghosh et al. (2002) research stone weight means reported between 0.06- 1.9. In present study, means of fruit weights were varied from 0.79 to 4.8 in Charmazoo and Gedaige ecotypes, respectively. Sivakov et al. (1988) reported that the means of fruit weights were varied from 5.72 to 10.45 in different *Ziziphus jujuba* Mill. genotypes. According to the other researches means of fruit weights of *Ziziphus jujuba* Mill. genotypes are found between 10 to 29.34 g (Kundi et al., 1989; Gao et al., 2003; Liu et al., 2004; Prasad, 2005; Jiang et al., 2006). Small fruits have high contents of vitamin C and TSS amounts compare to the large fruits (Sivakov et al., 1988; Kundi et al., 1989; Gao et al., 2003; Chen et al., 2006), this paper's results are in agreement with these studies. Also, cultivars with small fruits are suitable for nut products (Gao et al., 2003).

Table 3. Means comparison of vegetative traits in Ziziphus jujuba Mill. ecotypes

Ecotype	Stem annual-growth (cm)	Thorn-length (cm)	Petiole-length (mm)	Leaf-width (cm)	Leaf-length (cm)
Aidarvish	11.46e*	1.6bc	1.33d	1.36f	2.5c
Charmazoo	13.7e	2.2a	1.96cd	1.73e	3.7bc
Igder	19.33cd	1.8ab	0.2e	1.53ef	2.86bc
Gedaige	13.1e	1.8ab	1.96cd	1.7e	3bc
Sefid Cheshme	20.96bcd	1.93ab	3.2ab	2.16cd	4.43ab
Seid Kalate	18.96cd	1.93ab	3.5ab	2.23bc	4.33ab
Masoum Abad	16.03de	1.9ab	2.7bc	2.1cd	3.7bc
Takhshi Mahale	21.53bc	1.06d	3.4ab	1.9d	4.43ab
Roshan Abad	24.6b	1.2cd	4.1a	2.46a	4.6a
Lemesk	30.7a	1.73ab	3.9a	2.43ab	5.6a

*Means with the same letter are not significantly different using LSD test.

The highest ratio of fruit flesh/stone was 15.54 which in similar studies these ratios were found about 16.22 (Sivakov et al., 1988; Kundi et al., 1989; Ecevit et al., 2008).

In present study the thorn lengths were between 1.06- 2.02 cm. In Khakdaman et al. (2007) study, thorn lengths were varied from 2 to 5 cm and they suggested that climate can affect this trait.

In present research the leaf length and width were varied between 25-56 and 13-22 mm, respectively. In similar research, the amounts of these traits were different between 19-30 and 9-17.5 mm, respectively (Khakdaman et al., 2007). Also, the amounts of stem annual growth in my study were varied between 11-30 cm and in similar study it reported between 13- 29.75 cm (Khakdaman et al., 2007).

Cluster analysis according to the means of studied characteristics divided ecotypes to five groups. This detachment was almost similar with grouping of ecotypes according to their geographical origins. Gao et al. (2009) found that 29 clones of *Ziziphus jujuba* Mill. divided into four groups based on genetic distance 8.5. Lemesk and Masoum Abad ecotypes were settled in separate individual groups whereas Aidarvish, Igder, Charmazoo and Gedaige ecotypes were grouped in a same cluster, Takhshi Mahale and Roshan Abad ecotypes were grouped in another same cluster and Seid Kalate and Sefid Cheshme were settled in another same cluster, too.

Significant variations were found among the studied ecotypes. The interested point is the effects of climate on vegetative and reproductive traits in different ecotypes. Different ecotypes from same climate conditions were settled in a same cluster. The same results were reported by Khakdaman et al. (2007). They suggested that the reasons may be related to the origins of ecotypes or existence of differences in morphological traits of seedlings after planting in the similar climate conditions.

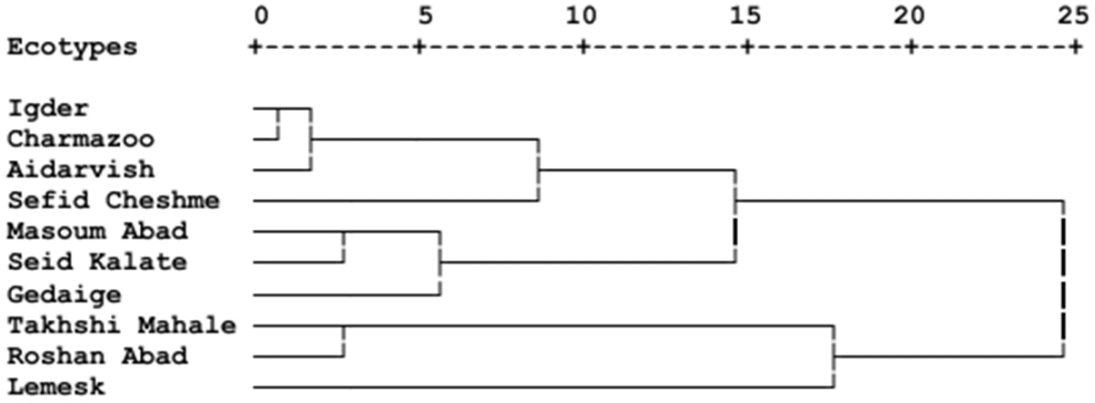

Figure 1. Dendrogram of grouping for *Ziziphus jujuba* Mill. ecotypes produced using Ward method.

Also, some ecotypes in similar climates show differences in reproductive characters that can be because of variations in pollination system (high rate of cross compatible) of *Ziziphus jujuba* Mill. genotypes (Khakdaman et al., 2007). Therefore, diversity frequency and evolution velocity of different traits among the ecotypes could be different. However, the wider scope and more types of the variations would provide more chances for new cultivar selection of the jujube (Liu, 2006). The climate has huge effects on the agromorphological traits of ecotypes for example ecotypes from west regions of the province (with high rate of rain) had different vegetative characteristics in comparison with the genotypes from the east regions of the province (with low rate of rain). Samples from humid regions had the lowest number and smallest thorns whereas samples from arid regions showed the high number of thorns with large size. This result is in agreement with Khakdaman et al. (2007) reports.

Conclusion

Variation provides us the possibility for high-quality genotypes selection. In conclusion,

the studied ecotypes of *Ziziphus jujuba* Mill. are diverse and variations among them are high. This provides a good germplasm for breeding objects of this species, especially for table and medicine objectives with good adaptations for climatic and soil conditions of Iran. Also, the ecotypes were separated according to their geographic origin. So it can be concluded that the climate effects is very important for existence of qualitative and quantitative characteristics of jujube. In addition, conservation of these ecotypes is highly recommended.

Acknowledgements

This project was carried out by financial supports of Seed and Plant Improvement Institute. I wish to thank Dr Nouri Nia for facilities.

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