Morphological Characterization of *Ficus religiosa* Genotypes in Iran by Multivariate Analysis

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Additional index words: bodhi, quantitative character, genetic resources, correlation coefficients, principal component analysis, cluster

Abstract. *Ficus religiosa* L. is one of the most popular species in the Moraceae family that is known as a multipurpose forest tree species because of its medicinal, ornamental, and religious value. *F. religiosa* is an important tree in South Asia, and it possesses various common names such as peepal, bodhi, bo tree, and asvatha. This species as a traditional tree is broadly planted as a roadside tree, and it plays an important role as a medicinal tree in various diseases such as asthma, stomatitis, diabetes, inflammations, glandular swelling disorders, and wound healing. Because *F. religiosa* is characterized as a subtropical tree, it fully grew in southern parts of Iran. The morphological variation of 72 individuals of *F. religiosa* from six southern regions of Iran was investigated based on multivariate analysis. Our results indicated that the highest tree, leaf, and petiole lengths, as well as leaf width, were observed in Kish and Qeshm genotypes, whereas the Chabahar genotype had the lowest petiole length. Results of simple correlation analysis showed the existence of significant positive and negative correlations among some important parameters. The highest correlation was observed between leaf, tree, and petiole lengths. Populations were clustered in four groups. The Kish and Qeshm genotypes were closely related to each other and differentiated from the Chabahar genotype. The whole dataset was subjected to principal component analysis (PCA). PCA showed that the first two factor components explained 84.51% of the variation and the first factor component had the positive relationship with leaf, tree, and petiole lengths.

*Ficus* is the genus of 1000 species in the family Moraceae, mainly distributed throughout tropical and subtropical regions (Hesami and Daneshvar, 2018). Many of this species have ornamental value and are also used as medicinal plants. *Ficus religiosa* L. is a long-lived, large, fuel wood, medicinal, ornamental, and evergreen perennial tree with glossy green foliage, native to India, mainly found in Pakistan, Bangladesh, Ceylon, China, Burma, Thailand, and Iran (Hesami et al., 2017b; Hesami et al., 2018b; Singh et al., 2011). It is also known as a roadside tree and most frequently found near temples. Different parts of *F. religiosa* are extensively used in indigenous medicine, especially for their antibacterial (Pawar and Nabar, 2010), anticonvulsive (Patil et al., 2011), antidiabetic (Kirana et al., 2009), antineoplastic (Ballabh et al., 2008), wound healing (Ghosh et al., 2016), anti-inflammatory and analgesic (Singh et al., 2011), antimicrobial and antiviral (Cagno et al., 2015), antihyperlipidemic (Keshari et al., 2016), antioxidant (Pandit et al., 2010), immunostimulant (Mallurwar and Pathak, 2008), antiasthmatic (Vinutha et al., 2007), and anticancer activities (Sankar et al., 2014) and parasympathetic modulatory (Dwivedi et al., 2014), as well as nootropic effects (Bhangale et al., 2016). *F. religiosa* can be multiplied by various methods, such as seed or vegetative methods (Hesami et al., 2017a; Hesami et al., 2018a; Salmi and Hesami, 2016). Recently, the intricate taxonomy of *F. religiosa* and other related species was highlighted by Ferrer-Gallego et al. (2016). Some traditional techniques for identification and characterization of genotypes and species are mainly focused on phenotypic observations. Morphological traits are known as an efficient and applicable way for preliminary evaluation because they can be applied as a general method for evaluating genetic diversity among morphologically distinguishable individuals and their simple evaluation as well. Morphological features in accompany with multivariate statistical methods, such as PCA that was broadly used, and cluster analysis, are applicable means for screening individuals for many plants such as *Cerasus* subgenus (Khadivi-Khub et al., 2012), *Prunus dulcis* (Chalak et al., 2007), *Origaniun vulgar* (Andi et al., 2011), and *Punica granatum* (Sarkhosh et al., 2009). Also, multivariate methods can help to assess large datasets and resolve various phenotypic and genotypic evaluations into fewer more interpretable and more easily visualized groups (Hesami et al., 2017c).

The feasible details about the diversity of morphological features within and among various subspecies of *F. religiosa* may be applied in the delimitation of subspecies and also it can be used in breeding programs and conservation of this valuable plant. Therefore, this article explains morphological variable of *F. religiosa* in different regions of Iran that also could apply to understanding the taxonomy of this valuable plant.

Materials and Methods

A total of 72 individuals of *F. religiosa* were studied in their natural habitats from six regions of southern parts of Iran (Fig. 1). Twelve plants were sampled randomly from each population. Sampling locations and their geographic coordinates are shown in Table 1. The interval between samples was 300–500 m, whereas the pairwise distance between main regions was 300–600 km. The sampled stands were chosen to provide a maximum representation of the ecological conditions of the area.

The morphological study was conducted in Spring and Summer of 2016. Eight morphological traits were (flake thickness, fruit diameter, leaf length, leaf width, peduncle length, petiole length, tail-like tips, and tree length) evaluated in natural ecosystems. Morphological data were analyzed by SAS program for analysis of variance (ANOVA). Mean of values were compared using ANOVA and Duncan’s multiple range test. The simple correlation coefficient was calculated to indicate the relationships between the studied traits, and PCA was performed using the SPSS software. Morphological characteristics were measured to evaluate the Euclidean distance between all pairs of individuals using the NTSYS-pc software version 2.01. These distance coefficients were applied for constructing a dendrogram using the unweighted pair group method with arithmetic averages using the sequential, hierarchical, agglomerative, and nested clustering algorithm. Also, scatter plot of the first two meaningful principal components was created by PAST statistics software.

Results and Discussion

According to Table 2, the highest tree, leaf, and petiole lengths, as well as leaf width, were observed in Kish and Qeshm genotypes,
Table 1. Locations of studied *Ficus religiosa* genotypes.

| Genotypes | Province         | Altitude (m) | Longitude (decimal degrees) | Latitude (decimal degrees) | Temperature (°C) | Precipitation (mm) | Relative humidity (%) |
|-----------|------------------|--------------|-----------------------------|---------------------------|------------------|--------------------|----------------------|
| Ahvaz     | Khuzestan        | 16           | 48.6842° E                  | 31.319° N                 | 25.4             | 209.2              | 43                   |
| Bandar Abbas | Hormozgan   | 9.1          | 56.2808° E                  | 27.1685° N               | 27.0             | 176.1              | 65                   |
| Bushehr   | Bushehr         | 8            | 50.8203° E                  | 28.9234° N               | 24.7             | 268.0              | 65                   |
| Chabahar  | Sistan and Baluchestan | 20 | 60.6459° E                  | 25.2969° N               | 26.3             | 118.0              | 70.3                 |
| Kish      | Hormozgan       | 32           | 53.9868° E                  | 26.5325° N               | 27.1             | 169.6              | 60.6                 |
| Qeshm     | Hormozgan       | 10           | 55.8913° E                  | 26.8119° N               | 27.0             | 141.9              | 63.1                 |

Means followed by the same letter within columns are not significantly different ($P = 0.05$) using Duncan’s multiple range test.

Table 2. Morphological traits of *Ficus religiosa* genotypes in different regions.

| Genotypes | Flake thickness (cm) | Fruit diam (cm) | Leaf length (cm) | Leaf width (cm) | Peduncle length (cm) | Petiole length (cm) | Tail-like tips (cm) | Tree length (m) |
|-----------|----------------------|-----------------|------------------|-----------------|----------------------|--------------------|--------------------|------------------|
| Kish      | 2.46 a               | 1.36 ab         | 16.00 a          | 9.02 a          | 4.78 a               | 9.58 a             | 6.74 a             | 33.30 a          |
| Qeshm     | 2.26 b               | 1.11 c          | 10.80 bc         | 7.83 c          | 4.12 c               | 8.72 bc            | 5.88 bc            | 26.40 b          |
| Bandar Abbas | 2.31 ab          | 1.43 a          | 14.80 a          | 8.86 a          | 4.62 ab              | 9.14 ab            | 6.24 b             | 30.20 a          |
| Bushehr   | 2.21 b               | 1.46 a          | 9.60 c           | 8.30 bc         | 4.26 bc              | 7.50 d             | 5.76 c             | 20.20 c          |
| Ahvaz     | 2.45 a               | 1.11 c          | 12.40 b          | 8.15 c          | 4.78 a               | 8.64 bc            | 5.22 d             | 24.80 bc         |
| Chabahar  | 2.30 ab              | 1.23 bc         | 12.20 b          | 8.72 ab         | 4.58 ab              | 8.44 c             | 5.92 bc            | 23.40 b          |

Means followed by the same letter within columns are not significantly different ($P = 0.05$) using Duncan’s multiple range test.
and also Chabahar genotype had the lowest petiole length (Fig. 2).

Results of simple correlation analysis (Table 3) showed the existence of significant positive and negative correlations among some important parameters. The highest correlation was observed between leaf, tree, and petiole length.

Populations were clustered in four groups (Fig. 3). The Kish and Qeshm genotypes were closely related with each other and differentiated from the Chabahar genotype. The highest distance was observed between the Kish and Chabahar genotypes (Fig. 3).

According to Table 4, PCA showed that the first two factor components explained 84.51% of the variation.

The first factor component had the positive relationship with leaf, tree, and petiole lengths and the second one had a negative effect with flake thickness, leaf length, peduncle, petiole, and tree lengths (Table 5).

These results have a high paramount to the success of a breeding program that mainly depends on the availability of a broad genetic base. Also, the maintaining of genetic diversity is one of the most important objectives in preserving endangered and threatened plants (Akbari et al., 2018; Ebrahimi et al., 2012a, 2012b; Farajpour et al., 2011; Hesami and Daneshvar, 2016a, 2016b; Khadivi-Khub et al., 2012). The knowledge of genetic diversity among populations gives some essential details in the formulation of suitable management strategies for preserving (Francisco-Ortega et al., 2000; Milligan et al., 1994). First, it should be prohibited to keep efficient population sizes because the population size is a critical restoration consideration in endangered plants (Allendorf, 1986). Cruze-Sanders et al. (2005) recommended that preserving a proportion of the individuals in populations is of high paramount to secure the evolutionary potential and reproductive fitness of the species. Second, the construction of an in situ preservation area is a perfect way to secure wild F. religiosa genetic resources. It will result in the efficient preservation of their genetic resources and the evolution of the resources under natural conditions. Third, it is necessary to provide a long-term schedule to preserve existing natural populations to protect as much genetic diversity as possible. Fourth, ex situ preservation that mainly focused on seed harvest from various sources should be conducted to capture most of the genetic diversity existed among populations. As an important conventional medicinal plant, promoting domestication and cultivation of this wild resource is very essential both to satisfy market demand and secure the wild resource. Successful cultivation may decline the harvest of the wild genetic diversity of F. religiosa and contribute to the conservation of this important medical plant.

Conclusions

Iran has the national and international heritage of some valuable medicinal plants such as F. religiosa and it is necessary to schedule a long-term plan to identify and understand the growth habitat of this valuable plant. Our results indicated that the Kish and Qeshm genotypes of F. religiosa had the highest tree, leaf, and petiole lengths, as well as leaf width. Also, these two genotypes had a close relationship with each other in comparison with other genotypes. The knowledge of genetic diversity among populations gives some essential details in the formulation of suitable management strategies for preserving (Francisco-Ortega et al., 2000; Milligan et al., 1994). First, it should be prohibited to keep efficient population sizes because the population size is a critical restoration consideration in endangered plants (Allendorf, 1986). Cruze-Sanders et al. (2005) recommended that preserving a proportion of the individuals in populations is of high paramount to secure the evolutionary potential and reproductive fitness of the species. Second, the construction of an in situ preservation area is a perfect way to secure wild F. religiosa genetic resources. It will result in the efficient preservation of their genetic resources and the evolution of the resources under natural conditions. Third, it is necessary to provide a long-term schedule to preserve existing natural populations to protect as much genetic diversity as possible. Fourth, ex situ preservation that mainly focused on seed harvest from various sources should be conducted to capture most of the genetic diversity existed among populations. As an important conventional medicinal plant, promoting domestication and cultivation of this wild resource is very essential both to satisfy market demand and secure the wild resource. Successful cultivation may decline the harvest of the wild genetic diversity of F. religiosa and contribute to the conservation of this important medical plant.

Fig. 2. Pictures of (A) leaves, (B) fruits, (C) petiole, and tail-like tips of Ficus religiosa in this study.
Table 4. Principal component analysis of morphological traits.

| Traits          | Component 1 | Component 2 |
|-----------------|-------------|-------------|
| Flake thickness | 0.318669    | -0.457801   |
| Fruit diameter  | 0.1089      | 0.650876    |
| Leaf length     | 0.447711    | -0.0167795  |
| Leaf width      | 0.363317    | 0.316454    |
| Peduncle length | 0.343079    | -0.255207   |
| Petiole length  | 0.401719    | -0.177167   |
| Tail-like tips  | 0.326494    | 0.411636    |
| Tree length     | 0.410447    | -0.0197917  |

Table 5. Components weight of morphological traits.

| Component | Percent of variance | Cumulative percentage |
|-----------|--------------------|-----------------------|
| 1         | 4.92994            | 61.624                |
| 2         | 1.83104            | 84.512                |
| 3         | 0.955183           | 96.452                |

Fig. 3. The cluster analysis of six genotypes of Ficus religiosa L.

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