

Inter-population Morphological Diversity in *Tulipa humilis* Herbert (*Liliaceae*) in Iran

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Abstract

Morphological diversity was studied among 15 populations of *Tulipa humilis* by using multivariate statistical methods. In total, 53 quantitative and qualitative morphological characters were studied out of which only 20 were informative in separating different populations. Cluster analysis, ordination plot as well as plot of discriminant analysis supported the presence of a sub-species for *T. humilis* in Iran.

Keywords: Cluster analysis, Morphological diversity, Tulipa.

Tulipa humilis تنوع ریختی میان جمعیتی در Herbert (Liliaceae)

مسعود شید!یی استاد گروه زیست شناسی دانشکه علوم دانشگاه شهید بهشتی شیوی کارشناسی ارشد زیست شناسی دانشکه علوم دانشگاه شهید بهشتی

مكنده

تنوع ریختی میان جمعیتی در ۱۵ جمعیت rulipa humilis با استفاده از روش های آماری چند متغیره مطالعه شد. تعداد ۵۳ صفت کمی و کیفی در ابتدا با استفاده از منابع بررسی شدند که از این میان ۲۰ صفت در میان جمعیتهای مطالعه شده تنوع بالا داشتند و جداکننده جمعیتها از یکدیگر هستند. تجزیه خوشهای، دسته بندی جمعیتها بر اساس تجزیه به مولفههای اصلی و تجزیه تابع تشخیص وجود یک زیر گونه در dailish تایید می کند.

كليدواژهها: تجزيه خوشهای، تنوع ريختی، Tulipa humilis.

Introduction

Tulips (*Tulipa* L.) are regarded as economically important ornamentals, grown wild and also cultivated in many countries. Tulips originated in Eastern countries were introduced into Europe via Iran and Turkey (Wendelbo,1977; Matin, 1998). The number of *Tulipa* species occurring in Iran varies up to 23 according to different authors.

Although Tulips have been studied extensively throughout the world, a limited number of biosystematic studies are vailable from Iran (Sheidai et al. 2002b; Khanafshar et al. 2004).

The present paper considers inter-population morphological diversity of *T. humilis* of Iran for the first time, trying to reveal if such differences have led to the formation of any taxonomic group below the species level.

Materials and Methods

Plant material

Fifteen populations of *T.humilis* were studied for their morphological diversity. Details of the localities and the voucher numbers are presented in Table 1. For the morphometric analyses, at least five plants were studied. Voucher specimens are deposited in TARI, IRAN and the Herbarium of Shahid Beheshti University (HSBU).

Morphometry

In total, 53 quantitative and qualitative morphological characters were studied (Table 2). Characters were selected on the basis of those reported by Van Raamsdonk and Varies (1995) as well as our own field studies. For the morphometric analyses, the mean of quantitative characters were used while qualitative characters were coded as binary/multistate characters. Variables were standardized (mean=0, variance=1) for multivariate statistical analyses (Sheidai et al., 2002a, b).

In order to group the populations with morphological similarities, cluster analysis using UPGMA

(unweighted paired group with arithmetic mean) and WARD (minimum variance spherical clusters) (Ingrouille, 1986) as well as ordination based on principal component analysis (PCA) were performed (Sheidai *et al*, 2002a). The squared Euclidean distance was used as the dissimilarity coefficient in a cluster analysis of morphological data.

In order to determine the most variable morphological characters among the populations, factor analysis based on principal components analysis (PCA) was performed.

In order to check the presence of a sub-species group among the populations studied, discriminant analysis, (DA) was performed (Lefebure and Vekemans, 1995) while, to check if there is any relationship between the geographical distance of the populations studied and their morphological diversity, the Mantel test was carried out (Brunell and Whitkus 1999). Statistical analyses were performed by using SPSS ver. 9 (1998).

Results and Discussion

Both the cluster analyses and an ordination plot based on PCA analysis of 15 *T. humilis* populations produced similar results (Figures 1 and 2), forming two major clusters. The first major cluster is comprised of two sub-clusters. The populations of the Kandovan tunnel, Polour, Imamzadeh-Hashem, Shemshak (all from Tehran Province), Golestan Kuh, Khonsar and Karkas mountain (all three from Isfahan Province) form the first sub-cluster. The populations of Touchal, Kolackchal, Tehran after Kandovan tunnel (all from Tehran Province) and Abadeh (Fars Province) form the second sub-cluster joining the members of the first sub-cluster at some distance due to their morphological differences.

The second major cluster is comprised of three populations from Ganjnameh, Alvand mountain (both from Hamedan, Province) and Barfkhaneh mountain (from Arak Province) which are placed far from the members of the first major cluster or group (Figures 1 and 2).

Table 1. Tulipa humilis population codes, their locality and Voucher specimens.

| Population code No. | Locality | Voucher, Collector and Herbarium |
|---------------------|-------------------------------|----------------------------------|
| 1 | Tehran, Imamzadeh-Hashem | Matin and Termeh 12887-IRAN |
| 2 | Isfehan, Knosar | Termeh et al. 12888-IRAN |
| 3 | Tehran, Polour | Matin and Termeh 12884-IRAN |
| 4 | Tehran, pro-Polour | Ershad and Reidel 12885-IRAN |
| 5 | Tehran, after Kandivan tunnel | Reidel and Habibi 14245-IRAN, |
| 6 | Tehran, Kandivan tunnel | Matin and Termeh 12882-IRAN |
| 7 | Hamedan, Ganjnameh | Bidari. 99125-SBU |
| 8 | Tehran, Touchal | Zojajifar 99131-SBU |
| 9 | Isfehan, Karkas mountain | WeendelboandForoughi 1432-TARI |
| 10 | Tehran, Shemshak | Weendelbo 17213-TARI |
| 11 | Tehran, KolaKlchal | Weendelbo and Kobham 17213-TARI |
| 12 | Arak, Barfkhaneh mountain | Weendelbo and Assadi 16476-TARI |
| 13 | Fars, Abadeh | Iranshahr 12931-IRAN |
| 14 | Hamedan, Alvand mountain | Fourooghi 17324-TARI |
| 15 | Isfahan, Golestan kuh | Weendelbo and Assadi 16395- TARI |

In order to determine the most variable characters among the populations studied, factor analysis based on PCA was performed revealing that the first four factors comprise about 81 % of total variation. In the first factor with about 41 % of total variation (Table 3), characteristics such as width of the lowest leaf and second leaf, width of the inner tepal, color of the anther, pollen and filament as well as tunic type possessed the highest positive correlation (>0.75).

In the second factor with about 21% of total variation, characteristics such as length of the lowest leaf, outer tepal, inner tepal and filament possessed the highest positive correlation (>0.60). The stem length and bulb width possessed highest positive correlation (>0.60) in the third and fourth factors. Therefore, these are the most variable morphological characters among the *T. humilis* populations studied. It is interesting to note that the characters of the

first PCA axis separates members of the second major cluster from the other populations and, as

already stated, these characters are both quantitative and qualitative. They therefore support an earlier

Table 2. Morphological characters and their coding range.

| | Stem length | Cm | Cm |
|----|--------------------------------------|------------------------------|-----|
| 2 | Stem pubescent | (y/n) | 0-1 |
| 3 | Number of leaves | | N |
| 1 | Length of lowest leaf | Cm | Cm |
| 5 | Length of second lowest leaf | Cm | Cm |
| 5 | Width of lowest leaf | Cm | Cm |
| 7 | Width of second lowest leaf | Cm | Cm |
| 3 | Leaf with deviating margin color | (y/n) | 0-1 |
|) | Leaf margin color | 1-like blade 2-red 3-white | 1-3 |
| 10 | Leaf pubescent | (y/n) | 0-1 |
| 11 | Leaf margin ciliate | (y/n) | 0-1 |
| 12 | Lowest leaf form | 1-crisp 2-falcate 3-straight | 1-3 |
| 13 | Second Lowest leaf form | 1-crisp 2-falcate 3-straight | 1-3 |
| 14 | Uppermost leaf form | 1-crisp 2-falcate 3-straight | 1-3 |
| 15 | Lowest leaf undulation | (y/n) | 0-1 |
| 16 | Second Lowest leaf undulation | (y/n) | 0-1 |
| 17 | Color of outer tepal at abaxial side | 1-red 2-yellow 3-orange | 1-8 |
| | | 4-white 5-purple 6-pink | |
| | | 7-silvery 8-coppery/violet | |
| 18 | Color of outer tepal at adaxial side | 1-red 2-yellow 3-orange | 1-8 |
| | | 4-white 5-purple 6-pink | |
| | | 7-silvery 8-coppery/violet | |
| 19 | Tepal with deviating margin color | (y/n) | 0-1 |
| 20 | Color of inner tepal at abaxial side | 1-red 2-yellow 3-orange | 1-8 |
| | | 4-white 5-purple 6-pink | |
| | | 7-silvery 8-coppery/violet | |
| 21 | Color of inner tepal at | 1-red 2-yellow 3-orange | 1-8 |
| | adaxial side | 4-white 5-purple 6-pink | |
| | | 7-silvery 8-coppery/violet | |
| 22 | Length of outer tepal | Cm | Cm |
| 23 | Width of outer tepal | Cm | Cm |
| 24 | Length of inner tepal | Cm | Cm |
| 25 | Width of inner tepal | Cm | Cm |
| 26 | Outer tepal blotch | 1-absent 2-black/dark purple | 1-5 |
| | | 3-purple 4-brown/violet | |
| | | 5-yellow | |

| 27 | Inner tepal blotch | 1-absent 2-black/dark purple | 1 |
|----|---------------------------------------|----------------------------------|-----|
| | | 3-purple 4-brown/violet | |
| | | 5-yellow | |
| 28 | Tip of outer tepal pubescent | (y/n) | 0- |
| 29 | Margin of outer tepal pubescent | (y/n) | 0- |
| 30 | Midrib of outer tepal pubescent | (y/n) | 0- |
| 31 | Tip of inner tepal pubescent | (y/n) | 0- |
| 32 | Margin of inner tepal pubescent | (y/n) | 0- |
| 33 | Midrib of inner tepal pubescent | (y/n) | 0- |
| 34 | Occurrence of yellow/white margin | (y/n) | 0- |
| | around blotch | | |
| 35 | Tip of outer tepal form | 1-acuminate 2-mucronate | 1- |
| | | 3-obtuse | |
| 36 | Filament length | Cm | C |
| 37 | Anther length | Cm | C |
| 38 | Anther color | 1-yellow 2-violet | 1- |
| | | 3-green/ purple | |
| 39 | Pollen color | 1-yellow 2-violet/purple | 1- |
| | | 3-green | |
| 40 | Filament color contrasting with | 0-similar 1-deviating | 0- |
| | flower color | | |
| 41 | Ovary length | Cm | Ct |
| 42 | Stigma color | 1-yellow 2- brown | 1-: |
| 43 | Width of bulb | Cm | Cr |
| 44 | Tunic type | 1-coriaceous 2-papery | 1-: |
| | | 3-sub-coriaceous | |
| 45 | Color of bulb tunic | 1-brown 2-dark brown | 1-2 |
| 46 | Occurrence of hairs at upper part of | (y/n) | 0-: |
| | bulb tunic | | |
| 47 | Occurrence of hairs at middle part of | (y/n) | 0-1 |
| | bulb tunic | | |
| 48 | Occurrence of hairs at base of | (y/n) | 0-1 |
| | bulb tunic | | |
| 49 | Bulb tufted at top | (y/n) | 0-1 |
| 50 | Type/form of tunic hairs | 1-tomentose 2-sericeous at tunic | 1-4 |
| | | base and strigose at summit | |
| | | 3-short hair 4-long hair | |
| 51 | Occurrence of carpophore at the | (y/n) | 0-1 |
| | base of capsule | 250000000 | |
| 52 | Capsule length | Cm | Cm |
| 53 | Capsule width | Cm | Cm |

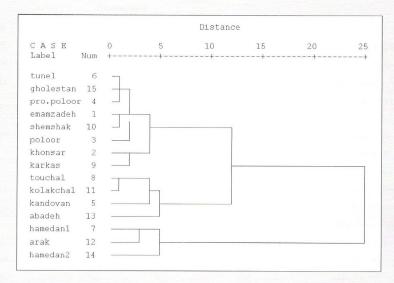
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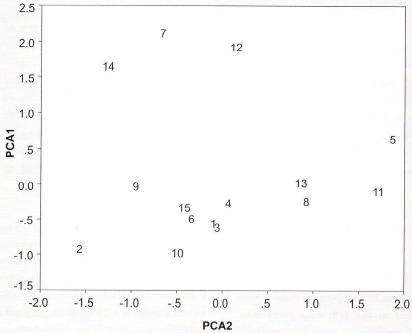
Table 3. PCA analysis of morphological characters.

| | Component | | | | |
|---|-----------|-----------|-----------|-----------|--|
| | 1 | 2 | 3 | 4 | |
| stem length | .446 | 2.542E-02 | .617 | 299 | |
| No.leaves | 365 | .313 | 540 | .363 | |
| Length of lowest leaf | .439 | .531 | 478 | .186 | |
| length of second | .473 | .634 | 498 | -5.70E-02 | |
| Width of lowest leaf | .768 | .223 | .144 | -6.70E-02 | |
| Width of second | .760 | .399 | .182 | 153 | |
| colour of flower | 713 | .384 | .397 | .113 | |
| center contrast colour of outside outer tepal | 713 | .384 | .397 | .113 | |
| length of outer tepetal | .552 | .702 | -1.27E-02 | 323 | |
| width of oute tepetal | .684 | .305 | .182 | .615 | |
| length of inner tepetal | .569 | .658 | -4.03E-02 | 289 | |
| width of inner tepetal | .822 | .290 | .348 | .244 | |
| colour of blotch | 916 | .298 | .135 | 3.177E-02 | |
| filament length | 137 | .748 | 448 | .202 | |
| anther length | .207 | .504 | .283 | 157 | |
| colour of pollen | .805 | 416 | -1.62E-02 | 6.779E-02 | |
| colour of anther | .805 | 416 | -1.62E-02 | 6.779E-02 | |
| colour of filament | .916 | 298 | 135 | -3.18E-02 | |
| length of ovary | -7.13E-02 | .853 | .316 | -4.24E-02 | |
| bulb width | .446 | -4.25E-02 | .472 | .665 | |
| tunice coriaceus | .916 | 298 | 135 | -3.18E-02 | |

study by Zojajifar and Sheidai considering these populations as members of a new sub-species of *T. humilis* namely *T. humilis* subsp. *Matini* (Zojajifar and Sheidai, 2001). However to give further support to such a suggestion, DA was performed and this will be explained in the following paragraphs. The variable characters of the second, third and fourth factors are all quantitative characters and only separate the members of the first major cluster or group (Figures 1 and 2). These variations might have

occurred due to environmental changes. Discriminant analysis of the morphological characteristics produced a single DA factor identifying the same PCA variable characters as did the important characters. The members of two major clusters identified earlier in a cluster dendrogram and PCA ordination were coded as two different groups and plotted against DA factors revealing distinctness of these two groups and supporting the sub-specific position of the Hamedan and Arak populations





Figures 1 and 2. WARD cluster analysis and PCA ordination of *T. humilis* populations. (Populations code as in Table 1).

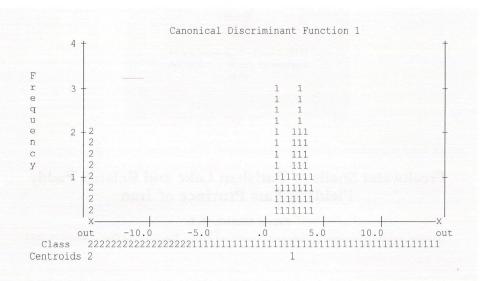


Figure 3. All-group stacked histogram of discriminant analysis among T. humilis populations. (codes: 1 = members of the first major cluster and 2 = members of the second major cluster of Figure 1).

(Figure 3). The predicted membership of each group was determined against the actual membership producing 100% correctness of grouping, further supporting the earlier results.

The Mantel test did not show any significant correlation between morphological differences among the populations and their geographical distance.

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