

Genetic Diversity of some Daffodil (*Narcissus* L. spp.) Genotypes from Turkey by Using SRAP Markers

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Abstract

Daffodil (*Narcissus* L. spp.) is one of the oldest ornamental flowers that have been cultivated from ancient times to nowadays. The species *Narcissus tazetta* L. and *N. serotinus* L. have spread out in different regions of Turkey and some other species have been naturalized. The purpose of this study was to characterize some daffodil populations that were collected from different regions of Turkey by using SRAP markers. Twenty-two daffodil populations of different species such as *N. tazetta*, *N. serotinus*, *N. pseudonarcissus* L., *N. poeticus* L. and *N. jonquilla* L. were used. Thirty-seven of 48 SRAP primer combinations showed scorable and polymorphic bands between samples. The similarity coefficients were used to construct a dendrogram by Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) using NTSYS-PC. Cophenetic correlation between ultrametric similarities of tree and similarity matrix were high ($r = 0.97$). The genotypes of *Narcissus* species were divided into 2 main groups. Although there was less genetic variation among *N. tazetta* species, even collected from different regions of Turkey, *N. poeticus*, *N. pseudonarsis*, *N. jonquilla* and *N. serotinus* had more genetic divergence among them. It can be concluded that genetic variation of *Narcissus* species can be used for daffodil breeding programs.

Keywords: flower bulb; genetic relationships; populations; SRAP

Introduction

Daffodils (*Narcissus* L.), members of family of Amaryllidaceae, are bulbous plants of great ornamental value that spread out in the Mediterranean basin (Rivera *et al.*, 2003). While several species are only known under cultivation (Wylie, 1952), the number of wild daffodils ranges between 40-60 species, depending on the species concept used (Jimenez *et al.*, 2009). It has been reported that around 60 *Narcissus* species spread out all over the world. But the highest diversity of daffodils has been seen in Iberian Peninsula. In addition to this, the species of *N. tazetta* does not only take place in Spain and North Africa but also in China and Japan (Mathew, 2002).

The species *N. tazetta* and *N. serotinus* are also spread out in different regions of Turkey (Davis, 1984). In Roman times, cultivation of daffodils took place in southern Anatolia (Izmir, Antalya) and in times of Ottoman Empire, many horticultural forms were cultivated in Istanbul. *Narcissus orientalis*, *N. byzantinus* and *N. constantinopolitanus* were all ancient forms of *N. tazetta*

which were moved to Europe from Istanbul (Baytop and Mathew, 1984). Although some different species of *Narcissus* are grown in Turkey, *N. tazetta* is the most commonly grown species for its attractive flower clusters and its special fragrance. Besides these species, there are also some species naturalized. One of them is the double form of *N. pseudonarcissus* naturalized in Belgrat Forest near Istanbul while *N. papyraceus* Ker Gawl. is naturalized in Izmir (Baytop and Mathew, 1984; Davis, 1984). Natural spread of wild species, naturalization of some species, cultivation since ancient times and being the source of some cultivars which were sent to Europe (Baytop and Mathew, 1984; Davis, 1984) reveals the genetic diversity of daffodils in Turkey.

Although *Narcissus* species have been cultivated for many centuries and spread out all over the world, there is still taxonomic intricacy due to recurrent process of long established cultivation and selection, and subsequent re-naturalization as well as the widespread occurrence of hybridization between species. However, *Narcissus* species have only few morphological differences that can be used for consistent taxonomic frameworks (Jimenez *et al.*, 2009). For the future breeding programs, the available genetic

pools and the conservation of the wild types are very important. Morphologic and molecular characterizations of the plant germplasm are important to make plant collection useful for breeders and conserve useful diversity of crop for utilization in crop improvement program (Upadhyaya *et al.*, 2002). Therefore, molecular marker technologies, providing robust and independent data have been used for molecular characterization and classification in different plant species. But there is restricted number of studies on molecular variation among *Narcissus* species (Perez-Baralles *et al.*, 2003; Barrett *et al.*, 2004; Tucci *et al.*, 2004; Graham and Barrett, 2004; Jimenez *et al.*, 2009). However there is no molecular characterization data on *Narcissus* species of Turkey.

Therefore, the aim of this study was to assess the molecular classification of *Narcissus* species in Turkey and to determine their genetic relationship. This would be helpful for taxonomic research as well as for genetic conservation and evaluation for further breeding programs. One of the PCR based markers, sequence-related amplified polymorphism (SRAP), was used to evaluate genetic diversity in this study. SRAP technique employs a combination of two primers, a forward primer of 17 bases, and a reverse primer of 18 bases, which consisted of preferential amplification of open reading frames (ORFs) and was developed for mapping and gene tagging in *Brassica* by Li and Quiros (2001). The observed polymorphism fundamentally originated in the variation of the length of introns, promoters, and spacers, both among individuals and among species (Li and Quiros, 2001). SRAP has been successfully used for evaluation of genetic diversity (Ferriol *et al.*, 2003), comparative genomics analysis and map construction (Li *et al.*, 2003).

Materials and Methods

The plant material consisted of 22 daffodil genotypes collected from natural distribution areas, from naturalized places in where daffodils plantation has been growing for years and from cultivated areas in different regions of Turkey (Fig. 1). The location data and flower type of the samples were given in Table 1.

Molecular analysis

DNA extraction

Genomic DNA was extracted from young daffodil leaves by using modified CTAB protocol according to Doyle and Doyle (1990). Leaf tissue was taken into 1.5 ml tubes and crushed using a small plastic bar in liquid nitrogen. CTAB (0.7 ml) buffer was added into the tubes, and then they were incubated at 65 °C for 60 minutes. After the incubation step 0.7 ml of chloroform + amyl alcohol mix was added and the tubes centrifuged at 11,000 rpm for ten minutes. The supernatant was transferred into a new tube and 0.7 ml of chloroform-amyl alcohol was added and centrifuged again. After centrifugation the supernatant was transferred into a new tube and DNA was precipitated in 2-propanol. The DNA was washed with 70% ethanol + ammonium acetate and then dried and dissolved in TE buffer. DNA concentration was measured by spectrophotometer and adjusted 10 µl/ng using TE buffer.

PCR protocol and gel analysis

In this study, in total 48 SRAP primer combinations (Table 2 and Table 3) were used. PCR was carried out in 15 µl reaction volumes containing: 2.5 mM of each primers, 250 µM of each dNTP, 1.5 µl of 10x PCR Buffer (Fermentas), 2 mM of MgCl₂, 1 unit of Taq polymerase and 25 ng of DNA template. The amplification reaction was carried out using a Thermo-cycler Mastercycler (Eppendorf-Germany) with an initial denaturation step at 94 °C for 3 min, followed by 5 cycles of 1 min at 94 °C, 1 min at 35 °C, 1 min at 72 °C, the PCR then continued for additional 29 cycles with an annealing temperature of 50 °C and finalized one cycle of 5 min at 72 °C. The PCR products were separated on 2.5% agarose gel in 0.5 TBE buffer at 90 volt for 4 h and photographed under UV light for further analysis. As a molecular standard, a 100 bp DNA ladder was used in order to confirm the appropriate SRAP markers.

Data analysis

Each band was scored as present (1) or absent (0) and data were analysed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software package (Rohlf, 2000). A similarity matrix was constructed based on Dice's coefficient (Dice 1945), which considers only one to one matches between two taxa for similarity. The similarity matrix was used to construct a dendrogram using the UPGMA (unweighted-pair group method arithmetic average) to determine genetic relationships among the germplasm studied. SIMINT module was used to compute a distance matrix. Then, a distance matrix was used to construct a dendrogram based on the UPGMA method. In order to see how well a cluster analysis represents the distance matrix, COPH module was used to transform the tree matrix to a matrix of ultrametric distances (a matrix of distances implied by the cluster analysis). MXCOMP module was used to compare these ultrametric distances and distance matrix produced for UPGMA analysis.

Results and Discussion

Out of the 48 SRAP primer combinations screened, 37 combinations were found as polymorphic and 3 combinations were monomorphic. The 37 polymorphic SRAP primer combinations produced total 154 polymorphic bands ranging size from 100 bp to 1500 bp.

Cophenetic correlation between ultrametric similarities of tree and very high similarity matrix ($r = 0.979$) was calculated.

The cluster was divided into 2 main groups with a genetic similarity of 32% (Fig. 2). The first cluster included species of *N. tazetta* and *N. serotinus* which display a genetic similarity that varied from 62% to 93%. The genetic distance of the other cluster, including *N. pseudomarcissus*, *N. poeticus* and *N. jonquilla*, varied between 60% and 82%. The relationships between the genotypes in the gene pool are also shown in two-dimensional and three-dimensional plots by using the data obtained from the factor analysis (Figs. 3, 4).

In the first cluster, the genetic distance among *N. tazetta* genotypes usually varied according to distance between their

Table 1. Collection data of 22 *Narcissus* populations included in the present study

| Number | Sample name | Taxa | The characteristics of population | Location | The characteristics of location | Flower type |
|--------|------------------------------|--|-----------------------------------|-------------------------|-------------------------------------|-------------|
| 1 | 1 <i>N. tazetta</i> | <i>N. tazetta</i> L. | Natural distribution | Samsun/Bafra | Natural habitats | Single form |
| 2 | 2 <i>N. tazetta</i> | <i>N. tazetta</i> L. | Naturalized | Istanbul/Beykoz | Private forest | Single form |
| 3 | 3 <i>N. tazetta</i> | <i>N. tazetta</i> L. cv Geranium | Naturalized | Istanbul/Beykoz | Private forest | Single form |
| 4 | 4 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Izmir/Seferihisar | Natural habitats | Single form |
| 5 | 5 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Izmir/Seferihisar | Agricultural area | Single form |
| 6 | 6 <i>N. tazetta</i> | <i>N. tazetta</i> subsp. <i>italicus</i> Ker Gawl. | Naturalized | Izmir/Odemis | Home garden | Single form |
| 7 | 7 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Muğla/Marmaris | Natural habitats | Single form |
| 8 | 8 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Antalya/Bahitli | Agricultural area | Single form |
| 9 | 9 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Mersin/Anamur Oren | Agricultural area, Natural habitats | Single form |
| 10 | 10 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Mersin/Tasucu | Agricultural area, Natural habitats | Single form |
| 11 | 11 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Mersin Erdemli | Natural habitats | Single form |
| 12 | 12 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Adana/Karatas | Agricultural area | Single form |
| 13 | 13 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Hatay/Isken. Arsuz | Agricultural area | Single form |
| 14 | 14 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Hatay /IskenderunBelen | Agricultural area, Natural habitats | Double form |
| 15 | 15 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Hatay /Kirikhan Karatas | Agricultural area | Single form |
| 16 | 16 <i>N. tazetta</i> | <i>N. tazetta</i> | Natural distribution | Diyarbakir/Hazro | Agricultural area | Single form |
| 17 | 17 <i>N. serotinus</i> | <i>N. serotinus</i> | Natural distribution | Muğla/Marmaris Beldibi | Natural habitats | Single form |
| 18 | 18 <i>N. serotinus</i> | <i>N. serotinus</i> | Natural distribution | Mersin/Anamur Oren | Agricultural area, Natural habitats | Single form |
| 19 | 19 <i>N. pseudonarcissus</i> | <i>N. pseudonarcissus</i> | Naturalized | Istanbul/Beykoz | Private forest | Double form |
| 20 | 20 <i>N. pseudonarcissus</i> | <i>N. pseudonarcissus</i> L. cv. Golden harvest | Cultivar variety | Izmir / Karaburun | Agricultural area | Single form |
| 21 | 21 <i>N. poeticus</i> | <i>N. poeticus</i> | Naturalized | Istanbul/Beykoz | Private forest | Single form |
| 22 | 22 <i>N. jonquilla</i> | <i>N. jonquilla</i> | Naturalized | Istanbul/Beykoz | Private forest | Single form |

Table 2. Primer sequences used for SRAP analysis of *Narcissus* populations from Turkey

| Primer | Type | Sequence (5'-3') |
|--------|---------|-------------------------|
| Em1 | Reverse | GAC TGC GTA CGA ATT AAT |
| Em2 | Reverse | GAC TGC GTA CGA ATT TGC |
| Em3 | Reverse | GAC TGC GTA CGA ATT GAC |
| Em5 | Reverse | GAC TGC GTA CGA ATT AAC |
| Em6 | Reverse | GAC TGC GTA CGA ATT GCA |
| Em8 | Reverse | GAC TGC GTA CGA ATT CAC |
| Em11 | Reverse | GAC TGC GTA CGA ATT CTA |
| Em12 | Reverse | GAC TGC GTA CGA ATT CTC |
| Em13 | Reverse | GAC TGC GTA CGA ATT CTG |
| Em14 | Reverse | GAC TGC GTA CGA ATT CTT |
| Em15 | Reverse | GAC TGC GTA CGA ATT GAT |
| Me1 | Forward | TGA GTC CAA ACC GGA TA |
| Me2 | Forward | TGA GTC CAA ACC GGA GC |
| Me3 | Forward | TGA GTC CAA ACC GGA AT |
| Me4 | Forward | TGA GTC CAA ACC GGA CC |
| Me5 | Forward | TGA GTC CAA ACC GGA AG |
| Me6 | Forward | TGA GTC CAA ACC GGA CA |
| Me7 | Forward | TGA GTC CAA ACC GGA CG |
| Me8 | Forward | TGA GTC CAA ACC GGA CT |
| Me9 | Forward | TGA GTC CAA ACC GGA GG |
| Me10 | Forward | TGA GTC CAA ACC GGA AA |
| Me11 | Forward | TGA GTC CAA ACC GGA AC |

Table 3. SRAP primer combinations which were used for molecular analysis of daffodil samples from Turkey

| | | |
|-----------|-----------|-----------|
| Em1-me11 | Em1-me3 | Em1-me5 |
| Em1-me4 | Em1-me6 | Em1-me9 |
| Em1-me10 | Em1-me7 | Em1-me8 |
| Em2-me1 | Em2-me2 | Em2-me3 |
| Em2-me4 | Em3-me1 | Em3-me2 |
| Em3-me3 | Em3-me4 | Em3-me5 |
| Em3-me7 | Em3-me11 | Em3-me12 |
| Em4-me1 | Em4-me3 | Em4-me6 |
| Em4-me7 | Em4-me8 | Em5-me11 |
| Em5-me12 | Em6-me1 | Em6-me3 |
| Em6-me6 | Em6-me7 | Em7-me8 |
| Em8-me9 | Em8-me11 | Em10-me9 |
| Em10-me13 | Em11-me11 | Em15-me1 |
| Em17-me11 | Em13-me2 | Em15-me6 |
| Em18-me11 | Em13-me6 | Em15-me10 |
| Em19-me11 | Em13-me11 | Em15-me11 |

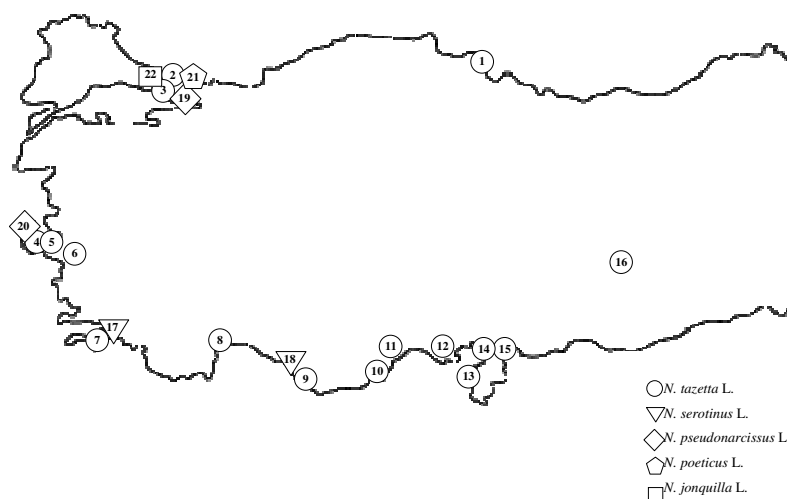


Fig. 1. The daffodil samples of different taxa which collected from different regions of Turkey

regional distributions. Genetically closest genotypes usually originate from closer regions (e.g. 4-5; 8-9; 10-11; 12-14-15). The genotypes of *N. tazetta* from south-west, south (7, 8, 9, 10, 11) and south (12, 14, 15) regions of Turkey had 84% genetic similarity (Fig. 1). The genotypes (genotype 4 and genotype 5) from Izmir located west part of Turkey were 22% genetically far from the genotypes from these south regions. The genetically closest genotypes (93%) were genotype 14 and genotype 15, even though one of them (genotype 14) was double flowered. The genotype 2 naturalized in Istanbul was surprisingly close (92%) to these genotypes from Hatay (14, 15). Although the high distance between two locations, it can be stated that the origin of the genotype 2 may be from Hatay. Genotype 13 was genetically 28% far from genotypes 14 and 15, even these 3 genotypes were from same region. However, Davis (1984) stated that some *N. tazetta* species between Iskenderun and Arsuz region (in Hatay) had different morphological properties such as dwarf plant with thin leaves. It can be stated that high genetic variation among genotypes in Hatay

region may have resulted from the morphological differences that Davis (1984) pointed out. The genetic similarity of the genotypes 1 and 16 from north and south (Samsun and Diyarbakır), which may be considered to be genetically far (26%) from other *N. tazetta* genotypes, was 84% (Fig. 2).

The genotype 3 (*N. tazetta* cv 'Geranium'), which is a multiheaded cultivar, had a genetically close relation with most of the *N. tazetta* genotypes (2, 7, 8, 9, 10, 11, 12, 14, 15), having genetic distance of 84-85%. This result confirmed that multiheaded cultivars, which are usually called Poetaz group, are an intersectional hybrid of *N. tazetta* and *N. poeticus* species (Doorenbos, 1954). Geranium cultivar was found to be genetically closer to *N. tazetta* than to *N. poeticus*. This may be due to the dominant effect of the *N. tazetta* parent in hybridization. However, Geranium cultivar is also morphologically much more similar to *N. tazetta* than *N. poeticus* with its multi flowered stem, and corona colour.

The genotype 6, *N. tazetta* subsp. *Italicus*, showed a genetic similarity ratio of 68% to *N. tazetta* species.

Narcissus serotinus species (genotype 17 and genotype 18) had about 62% of genetic similarity to *N. tazetta* species. Genetic closeness between *N. serotinus* and *N. tazetta* species may be expected a supportive result for their botanical classifications, where these two species belong to *Hermione* subgenus of *Narcissus* genus (Mathew, 2002).

In the second cluster, the genetic similarity of the genotype 19 (*Narcissus pseudonarcissus* from Istanbul) and genotype 20 (*N. pseudonarcissus*. cv. 'Golden' harvest from Izmir) was 82%, in spite of the morphological differences

(single and double flower forms) of these genotypes. Wild forms have been usually reported to have single flowers. Double types may have been produced by the duplication of the number of tepals, by changes involving the corona, or changes in the whole flower (Nunez et al., 2003). Also, Baytop and Mathew (1984) mentioned that a double formed flower of *N. pseudonarcissus* was naturalized in Istanbul. Therefore it can be stated that the genotype 19 may be originated from single flowered of *N. pseudonarcissus*. The genotype 22, *N. jonquilla* from Istanbul showed 60% of genetic similarity to genotypes of *N. poeticus* and *N. pseudonarcissus* species.

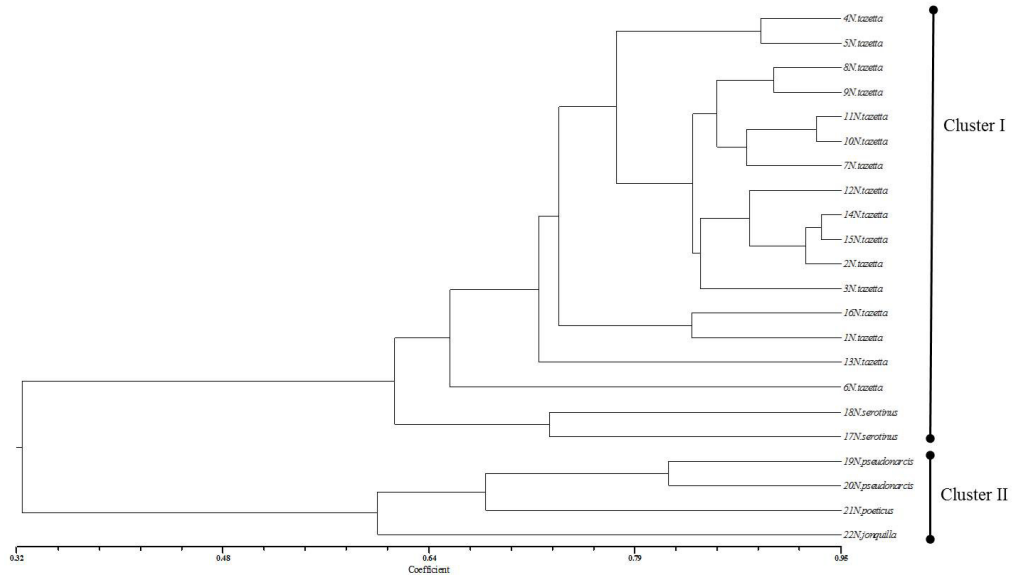


Fig. 2. UPGMA Cluster analysis of 22 daffodil samples from Turkey using 37 polymorphic SRAP combinations

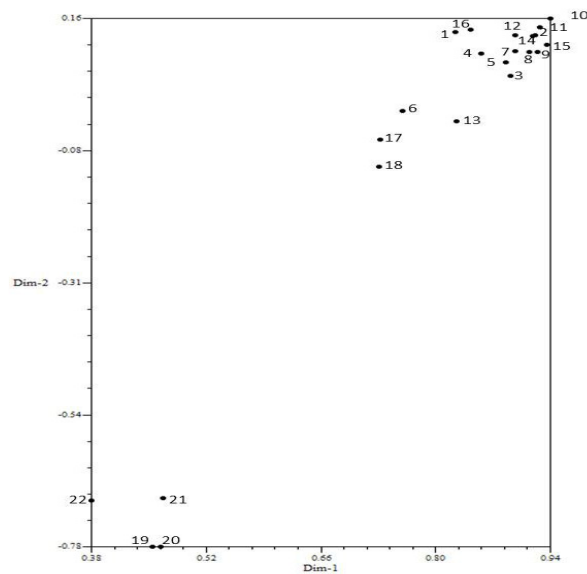


Fig. 3. Two-dimensional plane created by obtained axes from basic component analysis of relations between daffodil genotypes from Turkey

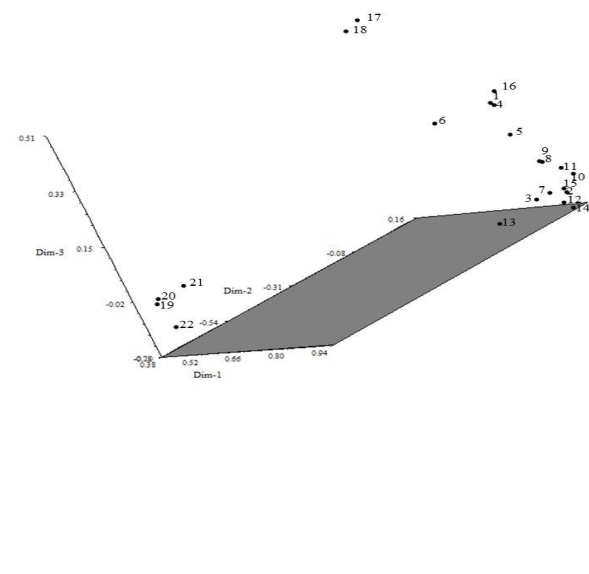


Fig. 4. Three-dimensional plane created by obtained axes from basic component analysis of relations between daffodil genotypes from Turkey

Conclusions

In conclusion, there was low genetic variation among *N. tazetta* species even collected from different region of Turkey. In contrast to this, *N. poeticus*, *N. pseudonarsis*, *N. jonquilla* and *N. serotinus*, showed more genetic divergence among them as an expected result as they are not closely related species to *N. tazetta*. Genetic distances of some of the *N. tazetta* samples from the other samples of the same species were also remarkable. This genetic background can be used for daffodil breeding program to widen genetic basis. Also, this paper is the first report on molecular classification of daffodil (*Narcissus*) species of Turkey. In this research we showed that SRAP markers can be used molecular characterization of *Narcissus* species and breeding purposes.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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