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GENETIC DIVERSITY ANALYSIS IN SNAPDRAGON (*ANTIRRHINUM MAJUS* L.) USING MORPHOLOGICAL AND MOLECULAR METHODS

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Abstract
Snapdragon (Antirrhinum majus L) is an important cut flower and cover plant grown worldwide because of their attractive flowers and long flowering duration. In this context, morphological and molecular characterization was carried out among 20 different accessions (17 local genotypes and 3 commercial cultivars) sampled from Kayseri region, where it is naturally found. The genotypes were evaluated for 15 different morphological characteristics such as leaf blade, flower, plant and time of flowering. Despite the commercial type has higher value (40 mm) than local genotypes for flower length, some local genotypes in terms have wider (11 mm) flower than commercial cultivar. Some local genotypes had higher plant heights than the commercial variety. The minimum value of the leaf length was measured at the number 19 (28 mm), while the maximum value was found at the number 4 (77 mm) in local genotypes. Inter-simple sequence repeat marker analysis (ISSR) indicated that the similarity coefficients were between 0.55 and 0.95. The cluster analysis divided the samples into two main branches. All local genotypes were clustered together in the first subgroup, while the second subgroup included the commercial variety alone. In conclusion, the local snapdragon genotypes were significantly different from the popular commercial cultivar, meaning that they can be used for broadening genetic background of the commercial varieties.

Keywords: Antirrhinum majus, ISSR, Snapdragon.

1. INTRODUCTION

Snapdragon (*Antirrhinum majus* L.) is a species of plants belonging to the genus *Antirrhinum* and is a member of family *Plantaginaceae* (Oyama and Baum, 2004). *Antirrhinum majus* has a flower with amazing shape and fabulous colors, commonly known as snapdragon or dog flower (Huxley and Griffiths Levy, 1992). It is herbaceous annual flowering plant in the Mediterranean region (Ramadan et al., 2013). *Antirrhinum majus* is extensively used as an ornamental plant and is one of the model species in genetic science (Mateu-Andres and De Pacol, 2005). *Antirrhinum majus* is produced seeds and it can be used in containers, borders, flower beds, various landscape plans, rockeries and herbaceous borders. The dwarf varieties are bushy and are very useful for window boxes and pots while the trailing types are used to hanging baskets (Bhargava et al., 2015). Flower petals are characterized by a highly different morphology manifested in a variety of shapes, sizes and a series of colors. It is thought that this diversity develops as adaptation to biotic pollinators, especially insects (Glover, 2011; Raczynska-Szajgin et al., 2014). In addition, *Antirrhinum* is an important cut flower crop grown throughout the world because of their showy, bright-colored flower heads available in a several colors (Ahmad and Dole, 2014). Also, this flower has a relatively

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long vase life (between 10 and 20 days (Jauhari and Singh, 2006). In recent years it has been reported that interest in the production of snapdragon plant has increased (Celikel et al., 2010).
Antirrhinum majus, seen as naturally in the Kayseri region, have colorful flowers. These plants are suitable for use in the refuges and ground cover plants. They are very attractive and actively grow up until the September by forming flowers. Research is needed for further information on the properties of this natural species. In this context, various morphological characterization studies were carried out. Molecular markers have contributed research on genetic variation and they are very useful for identification of variation (Sekerci et al., 2016). This study was performed for assessing genetic diversity and establishing genetic relationships by ISSR markers among different genotypes.

2. MATERIALS AND METHODS

1.1. Plant material

Seventeen *Antirrhinum majus* L. genotypes growing naturally in the Kayseri region and also three commercial cultivars are characterized by on-site observations in terms of their morphological characteristics. Samples from young leaves of these genotypes were used for molecular analyses.

1.2. Morphological characterization

The twenty genotypes were evaluated for 15 different morphological characteristics such as leaf blade (length, width, intensity of green color, leaf variegation), flower (type, length, width, shape, color, flower stalk length), plant (length, growth habit, attitude of shoots) and time of beginning of flowering (Table 1 and 2). Review of quantitative morphological parameter values is provided with means of three replicates.

1.3. DNA isolation and PCR amplification

Genomic DNA was isolated from young leaves of field grown plants using modified CTAB method (Doyle and Doyle, 1987). The quality and quantity of DNA isolated from these leaf samples was determined by agarose gel electrophoresis. The genomic DNA was subjected to PCR amplification using 15 ISSR primers (Table 3). PCR reaction was performed in a 15 ul volume containing 1.5 ul Taq buffer A (10 mM Tris–HCl, pH 8.3 with 15 mM $MgCl_2$), 1.2 ul of 2.5 mM dNTPs, 0.2 ul of 3 unit of *Taq* DNA polymerase, 2 ul (20 ng) of template genomic DNA and 1 ul (5 pM) each of ISSR primers. PCR reactions were run on a Bio-Rad C1000 thermocycler. Cycling conditions used for ISSR PCR amplification were as follows: initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min and a final extension step at 72°C for 5 min. The amplified products were resolved on 2% agarose gel at 70–80 V for 3.5–4 h, using 0.59 TBE (Tris-Boric acid-EDTA) buffer, visualized under UV light after staining with ethidium bromide and photographed using gel documentation system (Kodak EL Logic, 200).

1.4. Data analysis

Statistical analyses of the samples for morphological characters were carried out using Statistical Analysis System statistical software. Data analysis was carried out using one-way analysis of variance, and the comparative analyses were conducted using Duncan's multiple range test. A significance level of 0.05 was used for all comparisons.
For molecular analyses, only clear and reproducible DNA fragments were scored as 1–0 binary data matrix for the presence and absence of a band, respectively. The cluster analysis among the 20 genotypes of snapdragons was based on DICE' similarity coefficient using the unweighted pair-

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group with arithmetic average (UPGMA) in SAHN module nested in NTSYS-pc version 2.11 (Numerical Taxonomy Multivariate Analysis System, Exeter Software, Setauket, N.Y., USA). The total number of fragments (TNF), number of polymorphic fragments (NPF) and mean polymorphism (MP) for each primer combination are determined. Polymorphism ratios were calculated by using the formula number of polymorphic band/band number of total) X 100).

3. RESULTS AND DISCUSSIONS

Antirrhinum majus L, seen as naturally in the Kayseri region, have colorful flowers. These plants are suitable for use in the refuges and cover plants. Research is needed for further information on the properties of this natural species. Snapdragon is a species that attracts the attention of researchers in terms of potency, morphological characteristics, flower characteristics, vase life, etc. as ornamental plants. There are morphological characterization studies made by researchers (Ichimura et al., 2016; Alhajhoj et al., 2016; Nawaz et al., 2017). In this context, morphological characterizations of local *Antirrhinum majus* L. genotypes from Kayseri region were carried out in this study. The germination times were between June and September. The plant height was measured as 22 cm minimum in plant genotype 4 and 115 cm maximum in genotype number 6 (Table 2). General plant appearance and branching were determined. All genotypes had bushy type. Data of leaf size, leaf height, leaf width were presented in Table 2. The shortest leaf was determined in the number 19 (28 mm), while the longest leaf was measured in the number 4 (77 mm) in local genotypes (Table 2). There was considerable variation for leaf sizes among the local genotypes. Flower size and spike length were also investigated. The flower lengths of the genotypes ranged from 29 mm to 40 mm. Flower width was varied from 11 mm to 6 mm.

All genotypes selected from nature had different colors. Almost half of the genotypes had a multi-flower structure while the other half had a single flower structure (Table 3). Most genotypes had upright plant attitude of shoots (75%). Three of the genotypes showed early flowering, 7 of them medium and 10 of them showed late flowering. Similarly, the color intensity of the leaves was also variable. Seven genotypes had light leaf color, 5 have medium color and 8 have dark color leaves (Table 3). Flower density is an important issue for ornamental plants. It was determined that 6 of the genotypes constituted a large number of flowers. The snapdragon genotypes have very small seeds. The 100 seed weights determined within the scope of the study were between 0.010 g and 0.018 g.

The morphological and genetic studies carried out on Snapdragon plants are very limited, and particularly their ornamental potentials are largely unknown. Nawaz et al. (2017) have studied to reveal the morphological characteristics of the genus snapdragon. In their study, 24 exotics snapdragons have examined in terms of the plant and flower characteristics. The results of our study was similar to that of Nawaz et al. (2017).

Table 1. Mean values of some morphological characteristics of snapdragons

Genotype	Leaf width (mm)	Leaf height (mm)	Flower height (mm)	Flower: width (mm)	Flower stem length (mm)	100 seed weight (g)	Plant height (cm)
1	5.600g-h	30.67b	31.600d-f	7.8825e-h	19.750 fg	0.012 d	30 m
2	7.733c-g	39.44ab	32.655de	8.2350e-g	24.000ef	0.012 d	28 n
3	7.283c-h	39.95ab	31.640d-f	10.7600ab	18.000fg	0.010 f	27 o
4	5.768f-h	77.08a	28.925f	9.0200c-e	16.000f-h	0.010 f	22 r
5	9.303a-c	45.91ab	34.365de	11.3325a	41.500bc	0.011 e	62 ı

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6	10.505a	58.73ab	31.823ef	8.2600e-g	19.250fg	0.014 c	115 a
7	8.148c-e	42.67ab	32.880de	7.6700f-h	24.250ef	0.016 b	51 j
8	6.485d-h	42.61ab	36.183b-d	8.9400c-e	14.000g-ı	0.014 c	23 q
9	6.335d-h	40.66ab	32.870ef	10.9300a	11.750g-ı	0.018 a	69 f
10	9.130a-c	49.73ab	38.553ab	7.0625gh	73.500a	0.012 d	112 c
11	8.435a-d	48.66ab	38.573ab	8.4925d-f	43.500b	0.014 c	68 g
12	6.005e-h	48.13ab	35.893dc	8.2425e-g	31.750de	0.014 c	63 h
13	8.060c-f	40.46ab	38.183ab	7.5475f-h	20.750 fg	0.010 f	113 b
14	6.355d-h	44.14ab	38.063ab	9.6500dc	24.000ef	0.014 c	39 l
15	8.565a-e	28.06 b	30.095ef	5.9925 ı	7.000 hı	0.012 d	19 s
16	7.695c-g	29.77 b	36.813 bc	7.5675 f-h	5.250 ı	0.012 d	26 p
17	10.390ab	40.18ab	40.173a	9.0600c-e	18.250 fg	0.012 d	72 e
18	3.953ı	29.85b	37.603a-c	8.9600c-e	19.250fg	0.010 f	68 g
19	4.983ıh	28.47b	38.783ab	7.0150h	24.500ef	0.014 c	43 k
20	9.133a-c	52.44ab	36.450b-d	9.7600bc	33.500cd	0.010 f	79 d
LSD	**2.011**	**33.107**	**2.6145**	**1.0372**	**8.3952**	-	-
CV	**19.11222**	**52.56153**	**5.135387**	**8.507587**	**24.23856**	-	-

Table 2. Some morphological characteristic of snapdragons

Genotype	Flower color	Flower type	Growth habit	Attitude of shoots	Beginning of flowering	Leaf green color intensity	Flower density	Leaf variegation
1	white	double	bushy	upright	late	medium	low	absent
2	lilac	double	bushy	upright	late	light	low	absent
3	yellow-purple	double	bushy	upright	late	medium	high	absent
4	white-purple	double	bushy	upright	late	dark	high	absent
5	variegated	double	bushy	upright	medium	light	low	absent
6	purple	double	bushy	upright	early	dark	low	absent
7	variegated	single	bushy	upright	medium	medium	low	absent
8	variegated	single	bushy	upright	late	medium	medium	absent
9	dark pink	double	bushy	upright	medium	dark	low	absent
10	light pink	single	bushy	upright	medium	dark	medium	absent
11	pink	single	bushy	horizontal	late	light	medium	absent
12	yellow	double	bushy	upright	medium	dark	low	absent
13	white pink	double	bushy	semi upright	medium	dark	low	absent
14	cream	single	bushy	semi upright	late	medium	medium	absent
15	red (commercial)	single	bushy	semi upright	late	light	high	absent
16	white (commercial)	double	bushy	upright	early	light	medium	absent
17	purple (commercial)	double	bushy	upright	early	light	high	absent
18	dark purple	single	bushy	upright	medium	dark	high	absent
19	yellow-white	single	bushy	upright	late	dark	high	absent
20	purple-yellow	single	bushy	semi upright	late	light	medium	absent

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The UPGMA analysis was performed for estimating genetic diversity and relationships among the genotypes based on molecular data. Eighteen genotypes produced bands with ISSR markers. The obtained data were analyzed in NTSYS program. For molecular analyses of the samples, 13 ISSR primers out of 19 primers evaluated produced 86 scorable bands, of which 71 (83%) was polymorphic (Table 1). The most productive primer was $(GACA)_4$ yielding 11 bands while the least productive was $(GT)_6GG$ primer with 3 bands, averaging 6.6 bands per primer (Table 1).
Overall, the coefficients for genotypes ranged from 0.55 to 0.95. This study yielded the similar results with the study by Singh et al. (2014), who used 10 genotypes of the same species with RAPD primers and determined the similarity ratio as 0.43-1.00. The UPGMA dendrogram based on DICE' similarity matrix resulted in two groups at the similarity level of 0.55 in our study (Fig. 1). The most distinctive one was genotype 17, a commercial type. The genotypes 5 and 6; 7 and 14; 9 and 11 were very similar with the value of 0.90. Two snapdragon samples, 1 and 3, were clustered separately from the rest of other samples. The similar results were also observed between 10 and 20 (Fig. 1). Seemingly they have different genetic backgrounds.

Table 3. Details of amplification and polymorphic potential of ISSR markers in *Antirrhinum majus*

ISSR Primer	sekans (5'>>3')	TNF*	NPF*	MP (%)*
1	HVHTCCTCCTCCTCCTCCTCC	10	9	90
3	GACAGACAGACAGACA	11	11	100
4	DBDACACACACACACACA	7	6	85,7
5	AGAGAGAGAGAGAGAGT	8	7	87,5
8	AGCAGCAGCAGCAGCAGCG	8	3	37,5
11	GTGTGTGTGTGTGG	3	3	100
12	HVHCACACACACACACAT	7	5	71,4
13	VHVGTGTGTGTGTGTGTG	5	4	80
14	BDBCACACACACACACAC	4	3	75
16	CACCACCACCACCACCAC	4	3	75
17	CACACACACACACACAR	5	4	80
19	GAGAGAGAGAGAGAGAYG	7	6	85,7
20	TCCTCCTCCTCCTCCRY	7	7	100
TOTAL		86	71	82,5

*Total number of fragments (TNF), number of polymorphic fragments (NPF), Mean polymorphism (MP)

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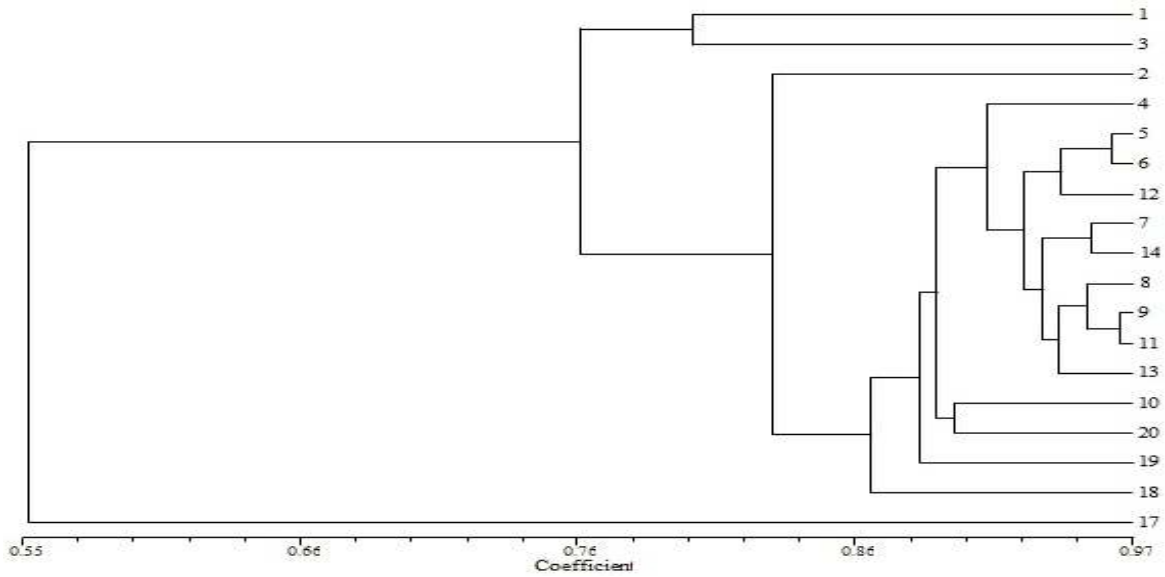

Figure 1. UPGMA dendrogram based on Dice similarity matrix

5. CONCLUSION

The morphological and genetic studies carried out on snapdragon plants are very limited, and particularly their ornamental potentials are largely unknown. Besides this, genetic diversity is important. In this study, ornamental and genetic characteristics of 20 *Antirrhinum* samples were investigated. All samples were distinguished from each other based on morphological and molecular data. Based on the observations, *A. majus* had good ornamental characteristics because of showy colorful flowers and considerable drought tolerance. They even occur in unintended areas where they have poor environmental conditions in Turkey. As assessed in this study, especially the flower density and flower color, flower size of plants showed considerable variation. This extended variation may offer great potential for Snapdragon breeding programs for ornamental use. The similarity coefficients for genotypes also ranged from 0.55 to 0.95, which may be beneficial to breeding programs. This study was carried out on snapdragon plants collected from a limited area, and future studies could be planned by collecting samples from a wider area. In addition, snapdragon's pharmacological properties can be examined in future studies.

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