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Application of UPOV Data for the Analysis of Genetic Variation in Rose Cultivars

Gi-Jun Kim[1], Young-Ha Song[2], Gwang-Yeon Gi[3], Seong-Tae Kim[4], Ja-Hyun Lee[2], and Tae-Ho Han[2,5]*

[1]Asiaseed Breeding Institute, Gwangju 464-863, Korea
[2]Division of Plant Biotechnology, Chonnam National University, Gwangju 500-757, Korea
[3]Horticultural Research Division, Jeonnam Agricultural Research & Extension Services, Naju 520-715, Korea
[4]National Institute of Horticultural & Herbal Science, Rural Development Administration, Suwon 440-760, Korea
[5]Institution of Agricultural Science and Technology, Chonnam National University, Gwangju 500-757, Korea

Abstract. The principal objective of this study was to estimate the availability of morphological data on the basis of the guidelines of the International Union for the Protection of New Varieties of Plants (UPOV) with regard to the identification of the rose germplasm. The correlation of morphological traits and random amplified polymorphic DNA (RAPD) marker data among 44 rose cultivars was assessed via a mantel test. Thirty eight phenotypes were employed for morphological analysis. Sixteenprimers were utilized for RAPD analysis, and these generated 225 polymorphic bands. The dendrogram based on the RAPD markersgrouped 44 rose cultivars according to their horticultural types. No significant correlation was observed between the morphological and RAPD marker data. We concluded that current UPOV traits could not be applied to studygenetic variation. Further studies onmorphological traits are required for the analysis of genetic variation among cultivars.

***Additional key words*:** breeding, cultivar, fingerprint, molecular marker, RAPD

Introduction

Rose is the most economically important ornamental crop world-wide. The genus Rosa consists of approximately 100 wild species and 20,000 commercial rose cultivars (Rajapakse et al., 2001). The number of chromosomes in the rose varies from 2n = 2x = 14 to 2n = 8x = 56. Also, frequent occurrences of spontaneous and artificial hybridizations have resulted in a complex genetic relationship among rose cultivars. Their incessant interspecific hybridizations, including artificial and spontaneous crossing, as well as extremely complex genetic mechanisms, render species diversity, and make it difficult to classify rose cultivars (Jan et al., 1999).

The emergence of the molecular marker technique has provided theefficient and effective identification of genetic diversity in plants. PCR-based marker techniques including random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have been continuously applied to the rose. These markers have been recognized as efficient tools for analysis of the genetic diversity of rose cultivars and species (Babaei et al., 2007; Debener et al., 2004; Esselink et al., 2003; Jan et al., 1999; Torres et al., 1993; Vosman et al., 2004). Notably, the RAPD marker technique has been shown to be reliable with regard to cultivars identification among rose (Debener et al., 1996, 1997; Jan et al., 1999; Millian et al., 1995; Mohapatra and Rout, 2005; Torres et al., 1993). The rose cultivars in this study were assessed via the previously selected RAPD markers, for later use as a reference. Another technique for cultivar identification involves the study of morphological traits in the rose. These UPOV guidelines have been developed in an effort to ensure the novelty of new cultivars. Thus, we assumed that this UPOV trait data might be utilized by breeders to estimate genetic diversity among cultivars. Traits that are less profoundly affected by the environment are used as the UPOV guidelines.

The objective of this study, then,was to assess the application of UPOV trait data in a cultivar diversity study. This was verifiedby comparing with the results of RAPD marker data, using the mantel test (Rohlf, 1998).

***Corresponding author:** wageningen@hanmail.net

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Materials and Methods

Plant Materials

The 44 cultivars utilized in this study consisted of 17 Hybrid Tea, 16 Floribunda, and 11 miniature cultivars nurtured at the Jeonnam Agricultural Research and Extension Service (JARES, Korea) (Table 1). Fresh leaves from the rose cultivars were collected and frozen in liquid nitrogen. The leaf powder was then stored at -70℃ until use. DNA was extracted from the leaf powder using DNeasy plant mini-kits (Qiagen, USA).

Table 1. Rose plant materials used in RAPD analysis.

Type	No. of accession	Common name	Country	Company
H	1	Amadeus	Germany	-
H	2	Las Vegas	Germany	Tantau
H	3	Goldengate	USA	J & P
H	4	Timeless	USA	-
H	5	Feria	France	Meilland
H	6	Maria Callas	France	Meilland
H	7	Royal Dream	Germany	-
H	8	Blue Bird	Germany	Tantau
H	9	Opulence	USA	J & P
H	10	Calibra	Germany	Kordes
H	11	Nobless	Germany	Tantau
H	12	Tiamo	Netherlands	-
H	13	Mister Lincoln	USA	Herbert C.swim O.L weeks
H	14	Sacha	Netherlands	P.kaster
H	15	Yellow Bird	New Zealand	McGredy
H	16	Kadinal	Germany	Kordes
H	17	Summer Lady	Germany	Tantau
F	18	Mariandell	Germany	Kordes
F	19	Diana	Germany	Tantau
F	20	Diadom	Germany	Tantau
F	21	Seventeen	USA	J & P
F	22	Night Star	USA	Dr.KeithW.zary
F	23	Gold Bunny	France	-
F	24	Red Velvet	Germany	-
F	25	Lampion	Germany	Tantau
F	26	Mary Devor	USA	Amling-DevorNusery
F	27	Matilda	France	Meilland
F	28	Margaret Merril	UK	-
F	29	Circus	USA	-
F	30	Spanish Sun	USA	-
F	31	Coco	Germany	-
F	32	Dallas	Germany	Kordes
F	33	Duftwolke	Germany	Tantau
M	34	Rosada	Spain	-
M	35	Cinderella	Netherlands	Jan de Vink
M	36	Yellow Meillandina	France	Meilland
M	37	Magic Carousel	USA	Moore
M	38	Clementine	Germany	Tantau
M	39	Little Mabel	UK	Fryer's Nursery
M	40	Pinocchio	New Zealand	Frank Bart Schuurman
M	41	Fetidcholie	-	-
M	42	Lydia	-	-
M	43	Pink Tango	-	-
M	44	Pink Charm	France	-

H: hybrid tea, F: floribunda, M: miniature.

Table 2. List of morphological characters measured by the International Union for Protection of New Varieties of Plants.

No.	Characters	No.	Characters
1	Plant: growth habit	20	Semi-double or double variety only: Flower: number of petal
2	Plant: height	21	Flower: diameter
3	Plant: width	22	Flower: view from above round
4	Young shoot: hue of anthocyanin coloration	23	Flower: side view of upper part
5	Prickles	24	Flower: side view of lower part
6	Prickles: shape of lower side	25	Flower: fragrance
7	Short prickles: number	26	Sepal: extensions
8	Long prickles: number	27	Petal: size
9	Leaf: size	28	Petal: color of middle zone of inner side
10	Leaf: green color	29	Petal: color of marginal zone of inner side
11	Leaf: glossiness of upper side	30	Petal: spot at base of inner side
12	Leaflet: cross section	31	Petal: color of spot at base of inner side
13	Leaflet: undulation of margin	32	Petal: color of middle zone of outer side
14	Terminal leaflet: length of blade	33	Petal: color of marginal zone of outer side
15	Terminal leaflet: width of blade	34	Petal: spot at base of outer side
16	Terminal leaflet: shape of base	35	Petal: size of spot at base of outer side
17	Flowering shoot: number of flowers	36	Petal: color of spot at base of outer side
18	Flower bud: shape of longitudinal section	37	Petal: reflexing of margin
19	Flower: type	38	Petal: undulation of margin

Morphological Data Description

The morphological traits of the rose cultivars in this study were generously provided from a previous investigations conducted by JARES. The morphological traits of the rose cultivars were measured in 38 phenotypes (Table 2). The phenotypic assessments were applied to the UPOV guidelines established for the rose.

RAPD Analysis

RAPD analysis was conducted twice for all the materials in order to verify reproducibility. For the RAPD analysis, fourteen 10 bp primers (Bioneer, Korea; Operon Technology, USA) and two 12 bp primers (BEX, Japan) were employed (Kim et al., 2006) (Table 3). The PCR reaction solution was prepared essentially as described by Kim et al. (2006). The PCR reactions were conducted with a PCT-200 DNA engine (MJ research, USA). The amplification program was performed as follows: 5 min at 95℃ for initial denaturation, 40 cycles at denaturation temperature of 95℃ for 1 min, annealing at 38℃ for 2 min, and extension for 2 min at 72℃. For the final extension, the samples were incubated for 7 min at 72℃. The amplified fragments were electrophoresed on 0.8% agarose gel containing ethidium bromide (EtBr) 0.5 $\mu g \cdot L^{-1}$ in 1 × TAE buffer at 140 V for 1hour, and the number of polymorphic and total bands were analyzed under UV light (Core bio, Korea). The sizes of the amplified DNA productswere determined using a 1 kb size marker (Bioneer, Korea).

Table 3. RAPD primers used for rose cultivar analysis and number of polymorphic bands.

Primers	Sequence 5'→3'	GC content (%)	No. of polymorphic bands
A-11	ACTGACCTAGTT	41.7	12
A-15	ATCGCGGAATAT	41.7	14
N-8005	GAAACGGGTG	60	18
N-8034	GCCGCTACTA	60	13
N-8038	GGTCCCTGAC	70	12
N-8045	CAAACGTCGG	60	13
N-8062	GACCGCAAGT	60	8
N-8072	CTTAGGGCAC	60	18
N-8079	GTGTGCCGTT	60	16
OPA-07	GAAACGGGTG	60	12
OPA-10	GTGATCGCAG	60	13
OPB-08	GTCCACACGG	70	15
OPB-17	AGGGAACGAG	60	16
OPD-12	CACCGTATCC	60	12
OPI-11	ACATGCCGTA	50	18
OPG-12	CAGCTCACGA	60	15

Data Analysis

The presence and absence of amplified bands were scored as 1 and 0, respectively. Morphological data were transformed into a binary form. The genetic dissimilarity matrix was calculated using the coefficients of Nei and Li (1979). Cluster analysis was conducted based on the unweighted pair group method with arithmetic averages (UPGMA) using NTSYS-pc version 2.0 software (Rohlf, 1998). Correspondence between the morphological and RAPD marker data of rose cultivars were conducted via mantel test using the NTSYS-pc version 2.0 software package (Rohlf, 1998).

Results

Genetic Relations among the Rose Cultivars Using RAPD Marker

44 rose cultivars were assessed with regard to genetic diversity. The total number of amplified bands was 225 (Table 3). The genetic dissimilarity of 44 cultivars varied from-0.06 to 0.43. The dendrogram was constructed via cluster analysis and generated two major groups. Group I and II were divided into 4 sub-groups, respectively (Fig. 1). Group I included Hybrid tea and Floribunda. Sub-group I-a was clustered with Hybrid tea. Sub-group I-b, c, and d were mixed with Hybrid tea and Floribunda. Insub-group I-d, the genetic similarity between 'Calibra' (HT) and 'Seventeen' (FL) was as high as 0.64. This confirmed that Hybrid tea and Floribunda roses share a close genetic background (Fig. 1). Group II was consisted of Floribunda and miniature roses. Sub-group II-f was clustered with Floribunda. Sub-group II-e, g, and h were grouped with miniature roses.

Consequently, the relationships of the 44 cultivars determined on the basis of their genetic distances were clustered precisely in the dendrogram constructed using the RAPD markers.

Morphological Analysis of Rose Germplasm

The application of morphological traits in the diversity study was assessed on the basis of the guidelines established by the International Union for the Protection of New Varieties of Plants (UPOV) with 38 established roses (Table 2). The similarity coefficients determined using morphological traits

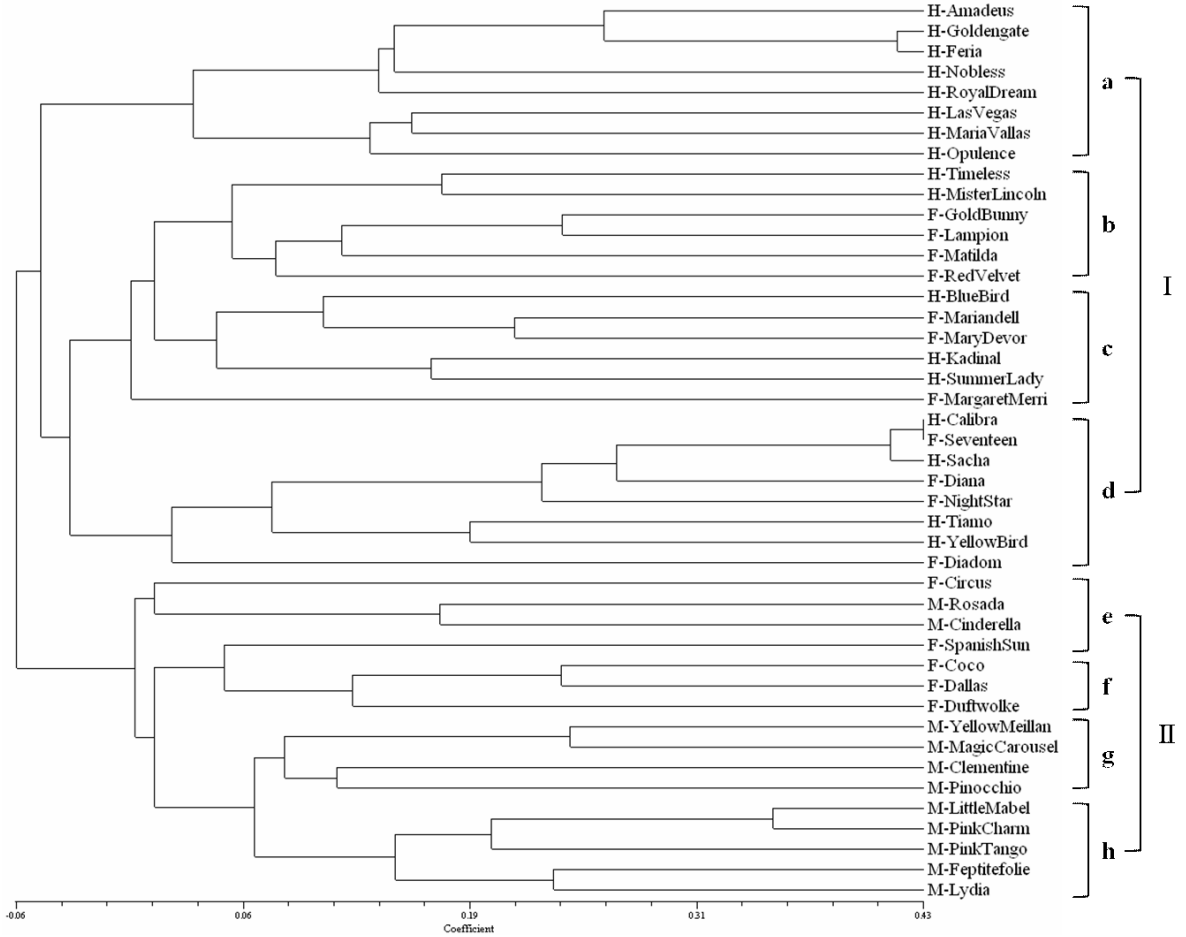

Fig. 1. Classification of the 44 rose cultivars constructed on the basis of RAPD data. H: hybrid tea, F: floribunda, M: miniature.

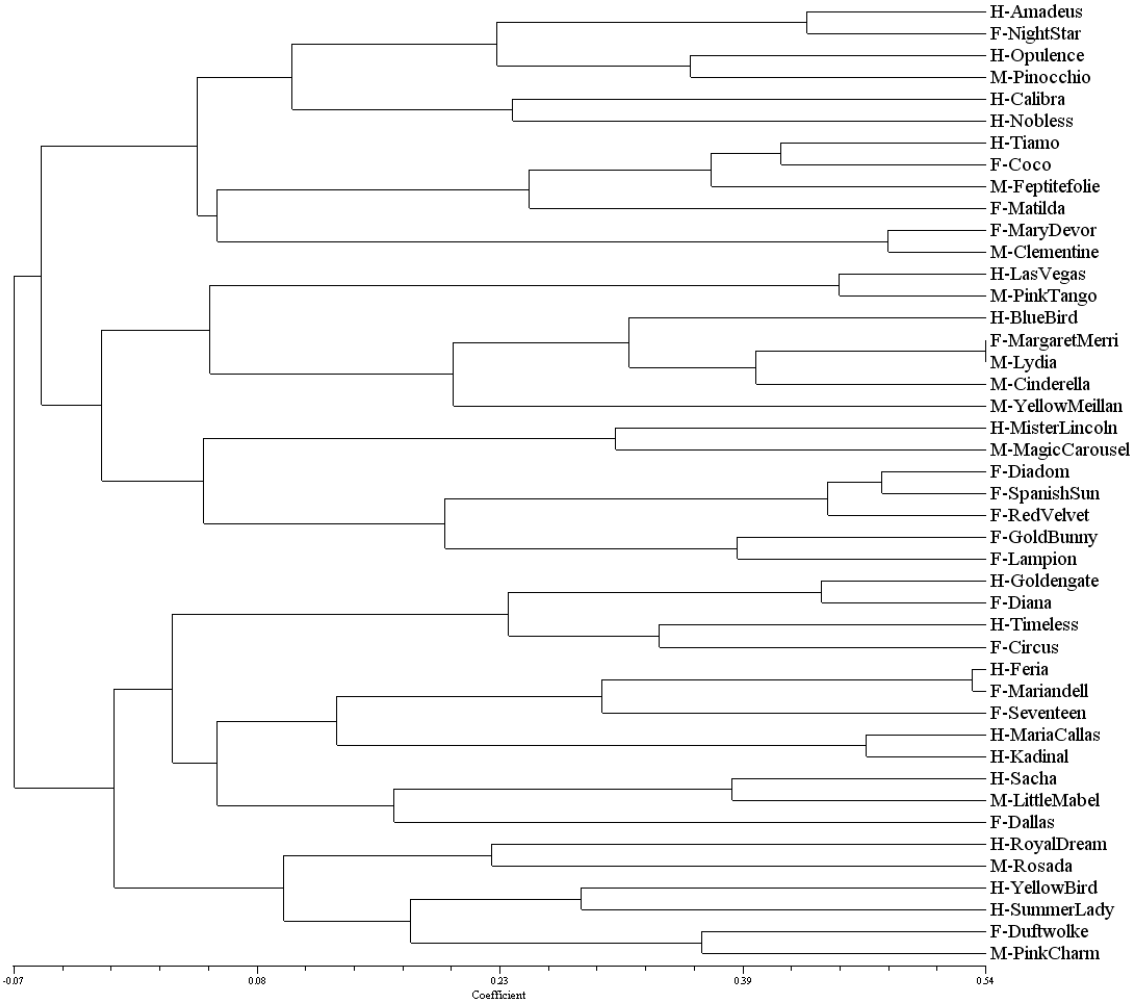

Fig. 2. Clustering of 44 rose cultivars based on morphological data. H: hybrid tea, F: floribunda, M: miniature.

was from -0.07 to 0.54 (Fig. 2). The similarity matrix was then employed in the construction of a dendrogram via the unweighted pair group arithmetic average method (UPGMA). 'Maria Callas' and 'Kadinal' showed a similarity of 0.47 among the 44 cultivars assessed in this study. Hybrid tea, Floribunda, and miniature cultivars were mixed within 44 rose cultivars (Fig. 2). Consequently, diversity analysis based on 38 morphological traits could not distinguish rose types in accordance with their genetic backgrounds.

Comparison between RAPD Markers and Morphological Data

Two dendrograms based on molecular markers and morphological traits data did not correspond to each other. The level of coincidence between two dendrograms could be measured via the estimation of the level of correlation of similarity coefficients. The correlation analysis was conducted with the mantel test using the NTSYSpc program (Fig. 3). This resulted in no relation (r = 0.05). The lack of a detectable correlation confirmed that any of the results could not be

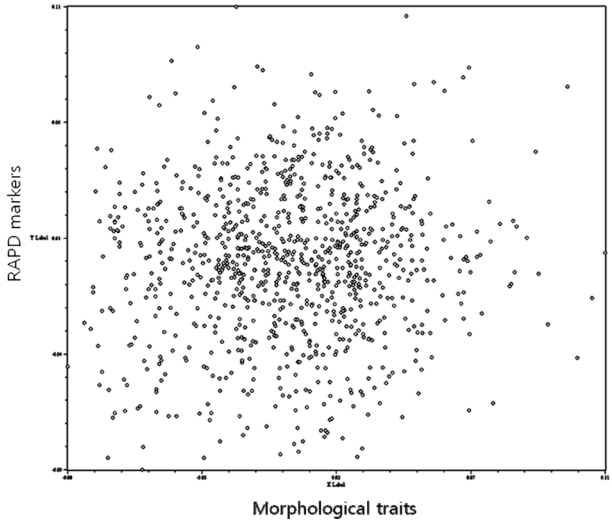

Fig. 3. Comparison between morphological and RAPD data using Mantel test of NTSYS-pc software.

used to assess their genetic diversity. We inferred from these results that the RAPD data was biologically applicable and, moreover,the traits must be investigated from the viewpoint

of their use in cultivar diversityanalysis.

Discussion

Breeding efficiency has been shown to be accelerated via the screening, investigation, classification, and evaluation of the germplasm (Diaby and Casler, 2003). Until the present, a classification system based on morphological traits has been generally utilized for the identification of rose cultivars. Although cultivar identification schemes based on morphological traits has certain limitations, including the overlap of morphological variations and the influence of environmental conditions (Jan et al., 1999), it also has advantages, including simplicity, speed, and low cost (Matínez et al., 2003). Molecular markershave been successfully applied to the identification of species and cultivars,principally because they are not affected by environmental conditions (Marić et al., 2004; Mohapatra and Rout, 2005).

In this study, RAPD markers could be used to distinguish clearly among 44 rose cultivars. The dendrogram indicated that Hybrid tea and Floribunda roses were clustered together (Fig. 1). The miniature roses were clustered separately from the Hybrid tea and Floribunda roses (Fig. 1). This result indicates that the Hybrid tea and Floribunda roses are genetically closer than the miniature roses.

These results were consistent with the previous results reported by Debener et al. (1996) and Mohapartra and Rout (2005). They reported that the Hybrid tea and Floribunda roses were very genetically similar, because the Hybrid tea roses were ancestors of Floribunda roses (Roberts et al., 2003). Also, the tiniest wild roses were identified as a major ancestor of miniature roses (Roberts et al., 2003) and the presence of Floribunda within miniature group II-e may be attributed to the origin of one of the ancestorsin a miniature type (Robert et al., 2003; Zlesak, 2006). Genetic relationship of various rose cultivars using RAPD markers was in agreement with rose taxonomy. Moreover, we reported that RAPD marker could be useful tool for rose pedigree and parentage determination in previous study (Kim et al., 2008).

The clustering results obtained on the basis of the morphological data evidenced a very complicated set of genetic relationships. The study of the pepper (Lefebvre et al., 2001) and grapevine (Matínez et al., 2003) yielded a better separated dendrogram based on the morphological characteristics between cultivars. In this study, the mantel test evidenced no correlation between the morphological and RAPD marker data. The low correlations between morphological and RAPD marker data have been similarly reported in the pepper (Kwon et al., 2005; Lefebvre et al., 2001).

Our experiment was predicated on the hypothesis that UPOV morphological traits could be objectively measured and utilized in studies of genetic diversity and cultivar identification. This would provide an alternative method by which genetic diversity measurements can be made in a simple, fast, and inexpensive fashion, as compared to molecular marker tools. However, we detected no correlation between morphological and RAPD marker genetic distances, as has been shown in other studies, including the pepper (Kwon et al., 2005; Lefebvre et al., 2001), wheat (Marić et al., 2004), and grapevine (Matínez et al., 2003).

Consequently, in order to identify cultivars of rose using UPOV morphological data, we must prepare objective data for statistical analysis via the removal of environmentally influenced morphological data or repeated data measurements that were conducted in many recurrent plants and under a variety of environmental conditions.

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