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Araştırma Makalesi (Research Article)

Molecular Characterization of Rose Genotypes *(Rosa sp.)* Based on RAPD-PCR

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Abstract
The genetic variation among different 47 rose genotypes, consisting of 27 modern garden types, 15 cut - roses including one bud mutant genotype from Dallas cultivar and 5 old garden roses were identified by using RAPD (Random Amplified Polymorphic DNA) molecular technique. Within 176 fragments (150-2070 bp) 133 fragments resulted in 75.4% polymorphism generated by 19 selective primers tested in previous studies. RAPD-PCR analyses led to efficiently differentiation as tree main groups within 47 genotypes comprising of modern garden roses, cut roses and old garden roses, which have been confirmed by correlation by UPGMA algorithm (Unweighted Pair Group Method Using Arithmetic Averages) and dendogram was also formed according to their horticultural groups. The results indicated that RAPD method is one of the effective molecular tools for identification and assessing of genetic relationship within rose cultivars grown under different horticultural groups.

Key Words: *Rosa sp*., RAPD, DNA isolation, genetic identification

Gül Genotiplerinin (*Rosa sp.*) RAPD-PCR Analizi ile Moleküler Düzeyde Tanımlanması

Özet
Aralarındaki genetik varyasyonun belirlenmesine yönelik olarak, 27 modern bahçe tipi gül, Dallas kesme gül çeşidinden tomurcuk mutasyonu ile elde edilen bir genotip de dâhil olmak üzere 15 kesme gül çeşidi ve 5 eski bahçe gülü, RAPD (Random Amplified Polymorphic DNA) tekniği kullanılarak moleküler seviyede tanımlanmıştır. Analizler için seçilen 19 primer, uzunlukları 150-2070 bp arasında değişen 176 bant üretmiş ve bunların 133 adedinin polimorfik olduğu belirlenmiştir (% 75,4). UPGMA yöntemi kullanılarak elde edilen RAPD-PCR analizlerinin gülleri genotiplerini tanımlamada; yetiştiricilik amaçları farklı olan modern bahçe gülleri, kesme gül çeşitleri ve eski bahçe gülleri arasındaki genetik ilişkileri ortaya çıkarmada kullanılabileceği belirlenmiştir.

Anahtar Kelimeler: *Rosa sp*., RAPD, PCR, genetik tanımlama

Introduction

Roses (*Rosa sp*.) are one of the most important traditional ornamental flowers due to their beauty, attractiveness and different usage areas. Rose species generally survive more than 35 million years in the world and its gene centre is Asia (Broertjes and VanHarten 1978). More than 200 species of the roses are present in the world and many of them are widely grown in the temperate zones of the Northern hemisphere (Kruessmann 1981, Cairns 1993). Although cultivating and breeding studies have been maintained in many years, breeding aimed to development of the desirable modern rose cultivars have been initiated at XXth century. Frequently spontaneous mutation and ploidy result in development hundreds wild species showing widely geographical distribution. Modern rose cultivars are generally triploid or tetraploid hybrids derived from 7-10 wild diploid rose species and a few tetraploid species. The exact numbers of the rose cultivars are unknown, whereas the number is estimated at more than 20000 rose cultivars in the world (Rajapakse et al. 2001).

Traditional identification and classification morphological characteristics of the roses are realized. Since each species of the genus *Rosa* and thousands cultivars have a wide overlapping range of morphological variations influenced by environmental conditions, morphological identification and classification of the rose species and cultivars are very difficult. Furthermore, when the genetic distance between varieties becomes smaller; identification and classification based on morphological traits become less efficient and are not adequate alone (Jan and Byrne 1999, Esselink et al. 2003).

Taxonomic studies of roses based on variation of polyphenolic compounds have been reported (Raymond et al. 1995). Isozyme markers were also used for

identification and classification of roses (Kim and Byrne 1994, Grossi et al. 1997). However, the small number of resolvable loci limits the utility of these markers. DNA-based molecular marker systems are efficient and informative for genetic analysis of roses due to DNA polymorphism indicated by markers and genetic profile is not affected by environmental conditions.

Several molecular marker systems, RFLP (Rajapakse et al. 2001), RAPD (Torres et al. 1993, Debener et al. 1996, Walker and Werner 1997, Jan et al. 1999, Ağaoğlu et al. 2000, Martin et al. 2001, Atienza et al. 2005, Kaur et al. 2007) SSR (Esselink et al. 2003) and AFLP (Zhang et al 2000; Baydar et al. 2004) have been used for genetic analysis of rose species, cultivars and rootstocks. RAPD markers have been widely used in many plant species in anthurium (Nowbuth et al. 2005), strawberry (Gaafar and Saker 2006), polyscias and schefflera (Rout et al. 2007), banana (Brown et al. 2009), olive (Sesli and Yeğenoğlu 2009) for identification, varietal analysis, population studies and genetic linkage mapping. RAPD markers are used to detect DNA polymorphism without requirement previous knowledge of the target genome (Williams et al. 1990). Also, RAPD markers are practical and can be effectively used for detection of genetic variation among rose genotypes (Debener et al. 1996, Jan et al. 1999).

Therefore, the aim of this study are also to characterize and identify genetic relationship within cultivated 47 rose genotypes selected from different horticultural groups including cut roses, modern garden roses and old garden roses using RAPD-PCR method.

Material and Methods

Plant material: The young leaves of 27 modern garden roses were supplied from the rose germplasm collection of Atatürk Central Horticultural Research Institute in Yalova and 15 cut-roses from traditional greenhouses around Yalova. 5 old garden roses were also included in RAPD analyses (Table 1). *R. foetida* which is old garden rose is one of the wild species grown in flora cultivating as garden rose of Turkey. The old garden species known as 'Van' is classified in *R. centifolia* (Baytop 2001). Another old garden rose species known as 'Isparta' is a very important variety of *Rosa damascena* in terms of oil production and medicinal properties in Turkey. Fresh leaves of 47 genotypes were stored at -20°C until DNA isolation.

DNA isolation: DNA extraction at sufficient amount and quality is very difficult from rose leaves because of secondary compounds. DNA isolation protocol with CTAB (Cationic hexadecyl trimethyl ammonium bromide) was modified from Doyle and Doyle (1988). Two times extraction with 2-mercaptoethanol was also done by addition an antioxidant and PVP bound phenolic compounds.

Table 1. The rose genotypes collected for molecular characterization by RAPD.

Garden Roses	13. Inter Flora	26. Sympathy	37. Jacarantha
1. Americane	14. Jaguar	27. Violeine	38. Marilyn Monroe
2. Antigone	15. Kabuki		39. Osiana
3. Ariane	16. Keberg	**Cut Roses**	40. Pirol
4. Ariosa	17. Laynd	28. Akito	41. Texas
5. Baby Baccara	18. Maria Callas	29. Arifa	42. Vandela
6. Baccara	19. Papa Meilland	30. Atache	
7. Cameo	20. Princess Margaret	31. Athena	**Old Garden Roses**
8. Carina	21. Queen Elizabeth	32. Black Magic	43. Isparta
9. Cleo	22. Salmon Perfection	33. Dallas	44. Van
10. Chrysler Imperial	23. Sophia Loren	34. Dallas BM*	45. Kishmir
11. Diamond	24. Soraya	35. Grand Gala	46. Miniature rose
12. Dr. Verhage	25. Super Star	36. Helmut Schmidt	47. *Rosa foetida*

*Dallas BM is a new pink variety not certificated. It is derived from Dallas red cut rose via bud mutation (personal communication with expert Kamil Gürsan).

100 mg young leaf tissue was grounded with liquid nitrogen and incubated at 65°C after 4X CTAB was added for 1 hour. Then homogenates were extracted with chloroform: isoamylalcohol (CIA, 24;1) and aqueous phase was transferred in a new tube after centrifuge. DNAs were precipitate with isopropanol and washed two times with ethylalcohol. The final pellet was dissolved in 200 µl TE buffer (10 mM Tris-HCl, 0.1 mM EDTA pH 7.5). All extraction stages were repeated using 1X CTAB. DNA concentrations

adjusted at 50 ng μl^{-1} were determined by spectrophotometer (Biowave S2100 Diode Array Spectrophotometer) and confirmed by % 0.8 agarose gel electrophoresis.

PCR amplification: 19 RAPD primers comprising of ten bases resulted in high polymorphism A01, A09, A11, A18, E04, E14, E19, F01, F06, F14, G11, G19, H06, H12, H15, H19, J04, T04, T19 (Operon Technologies, Alameda, California) were used for analysis. Amplification of the RAPD fragments were performed in a Appligene-Oncor Crocodile III thermocycle in 25 µl volume reaction buffer (10X PCR buffer, 2.5 mM $MgCl_2$, dNTPs each 0.2 mM, 20 ng μl^{-1} primer, 5U *Taq* DNA polymerase and 50 ng μl^{-1} template DNA). Thermocycle was programmed for 1 cycle of 4 min. at 94°C (initial denaturation), 45 cycles of 1 min. at 94°C (denaturation), 1 min. at 34°C (annealing), 2 min. at 74°C (extension), finally 1 cycle of 5 min. at 92°C (final extension). PCR products were subjected to electrophoresis in 1.5% agorose gel, visualized by ethidium bromide staining under UV transilluminator.

Data analysis: PCR reactions were repeated tree times and only reproducible bands were scored. Each polymorphic amplified fragment was scored as presence (1) and absence (0) and data were transcribed into binary format. Based on the matrix of genetic similarity, cluster analysis was done. The UPGMA method (unweighted pair group method with arithmetic averages) was used for clustering employing the NTSYS pc (Numerical Taxonomy and Multivariate Analysis System, version 1.8) program (Rholf 1994).

Results and Discussion

RAPD-PCR analyses performed with 19 selective 10-mer primers were used to characterize 47 rose genotypes. 133 fragments of total 175 bands showed 75.4% polymorphism. The number of bands related to primers varied from 5 (E14 and H12) to 15 (E04 and T19), on the other hand the number of polymorphic bands fluctuated between 2 (H12) and 13 (E04 and T19). The number of amplified fragments and their polymorphism ratios were shown in Table 2. The average number of polymorphic band for each primer was 7.0. The size of the amplified fragments ranged from 150 to 2070 bp and A01, E04, F01, F06, F14, G11, G19 and T19 primes gave the best results within rose genotypes *(*Figure 1).

Dendogram of the relative genetic distance between 47 rose genotypes was constituted of tree main groups (Figure 2). Group 1 involved modern roses were divided into tree divisions. Other cut roses except 'Texas', 'Jacarantha' and 'Pirol' composed Division A. Divisions B and C were exactly composed of modern garden roses. 'Dallas BM' is a novel pink variety derived from 'Dallas' red cut rose via bud mutation. The highest relative genetic similarity (0.955) was between these cut rose genotypes. 'Dallas' and 'Black Magic' which are the most cultivated varieties have almost exhibited same morphological traits as red cut roses and also showed closely relative genetic similarity based on dendogram (0.903).

Group 2 was composed of modern garden roses (Keberg, Kabuki, Maria Callas and Ariane), cut roses (Jacarantha, Pirol and Texas) and one of the old garden roses (Van). The genetic similarities between genotypes in Group 2 were less than genotypes in Group 1. 'Jacarantha' one of the trees cut roses and 'Ariane' located in Group 2, are known as early improved rose varieties. Although 'Pirol' and 'Texas' are classified cut roses, their plant habitus and flowering type are similar to wild types. Therefore these varieties were in Group 2. There is no more data on pedigree of other genotypes located in Group 2.

Relative genetic similarities between 4 old garden rose genotypes (Miniatur, Kishmir, *R. foetida* and Isparta) located in Group 3, were less than between genotypes of Group 1 and 2. *Rosa foetida* was suggested as one of the wild species grown in Turkey's flora and donor genotypes to be used in breeding program of the modern garden roses in Europe (Baytop 2001).

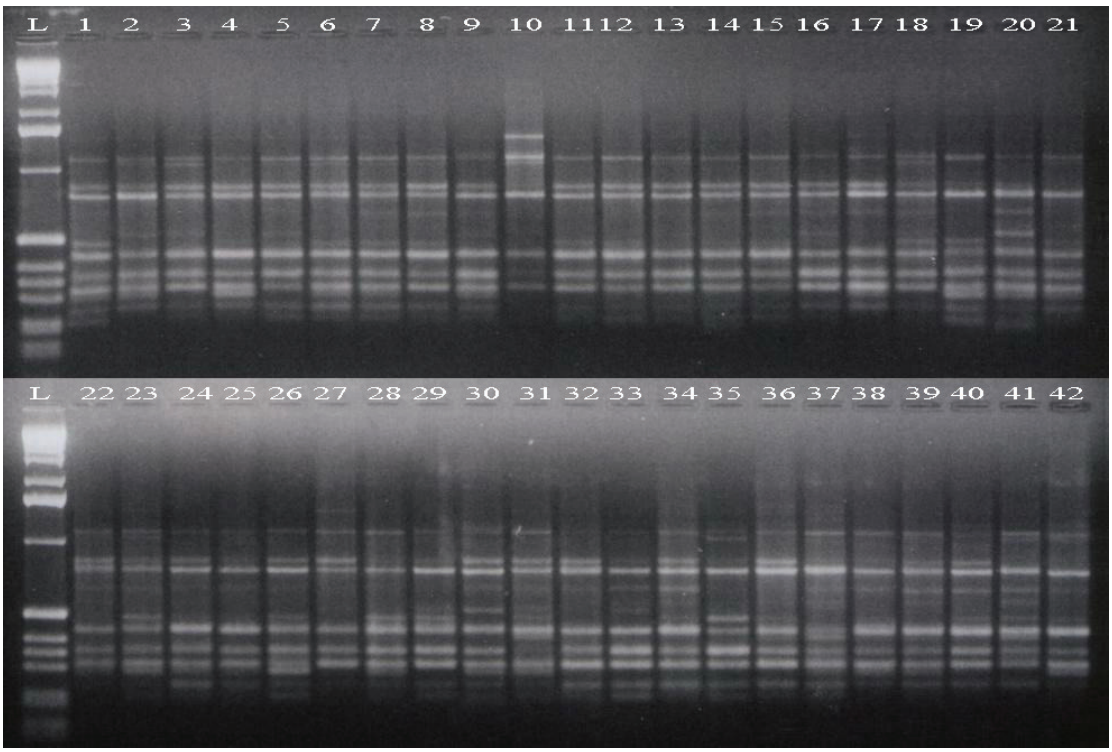

Figure 1. Amplified fragment petterns of first 42 genotypes generated from F06 RAPD primers (L: 1kb DNA size marker, Gibco BRL Inc.; Line 1-27, modern garden roses; Line 28-42 cut roses).

Table 2. The number of bands and polymorphism ratio generated by PCR amplification.

Primers	Visible bands (no)	Polymorphic bands (no)	Polymorphism (%)
A01	11	8	72.7
A09	7	4	57.1
A11	8	6	75.0
A18	8	6	75.0
E04	15	13	86.6
E14	5	3	60.0
E19	9	7	77.8
F01	10	8	80.0
F06	13	10	76.7
F14	12	10	83.3
G11	7	6	85.7
G19	11	9	81.8
H06	10	7	70.0
H12	5	2	40.0
H15	8	6	75.0
H19	8	7	87.5
J04	7	3	42.9
T04	7	4	57.1
T19	15	13	86.7
Total	176	133	75.4

RAPD data suggested a relationship between horticultural and molecular classification of the rose genotypes. Genetic differences assessed by RAPD-PCR analysis between cut and garden roses were associated with traditional classification. Identification by RAPD markers of different rose species located in *Caninae and Cinnamomeae* horticultural groups of subgenus of *Rosa* has also been reported (Debener et al. 1996).

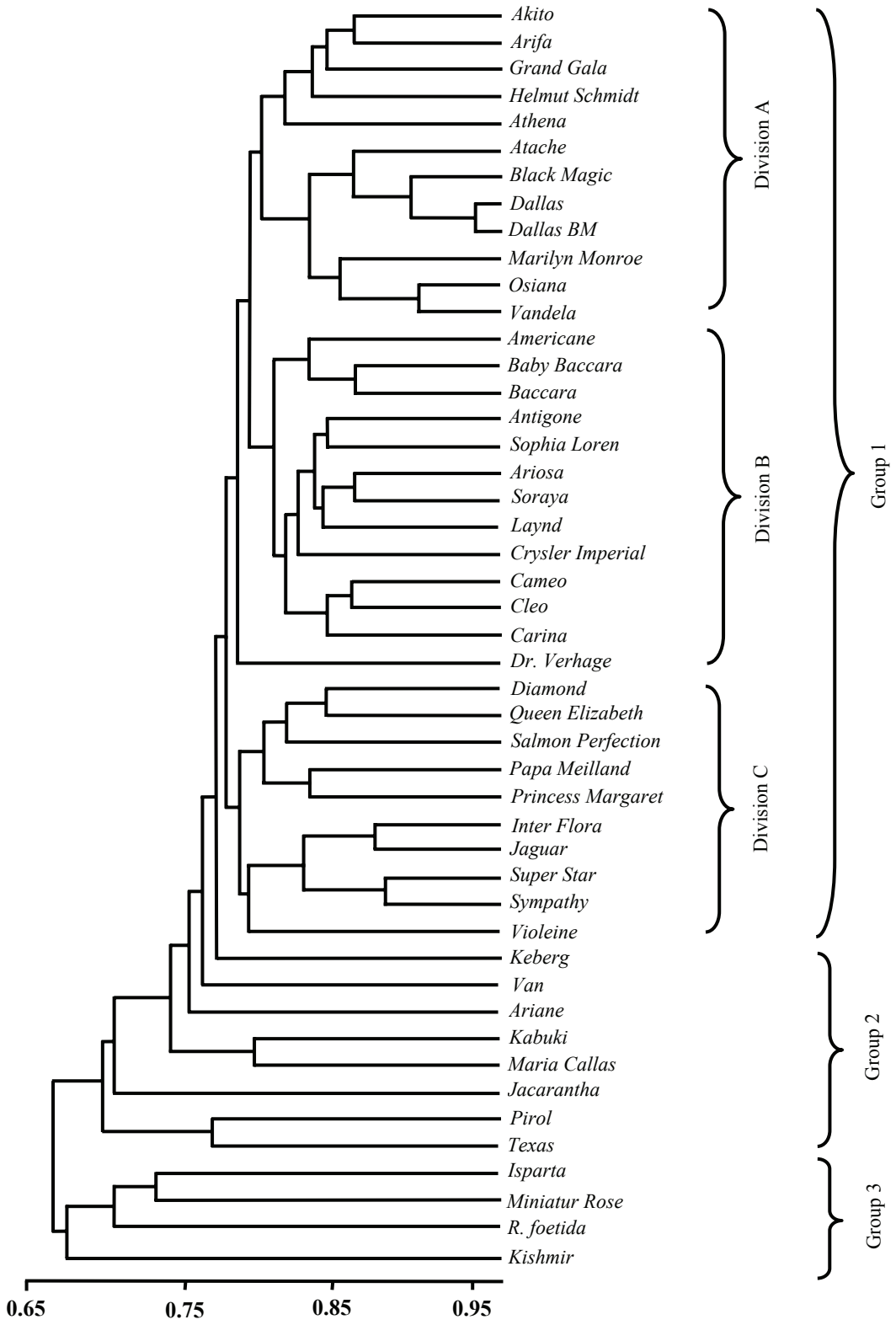

Figure 2. Dendogram of the relative genetic distance within 47 rose genotypes according to UPGMA cluster analysis.

In conclusion, RAPD-PCR method and the selected primers tested can be suggested as useful tools to identify rose genotypes, and precursor in assessing of the near genetic relationships within mutant genotypes, improved cultivars, old garden and/or wild rose species.

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