African Journal of Biotechnology Vol. 11(47), pp. 10650-10654, 12 June, 2012
Available online at http://www.academicjournals.org/AJB
DOI:10.5897/AJB10.1375
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Genetic diversity of rose germplasm in Pakistan characterized by random amplified polymorphic DNA (RAPD) markers

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Accepted 2 November, 2010

Random amplified polymorphic DNA (RAPD) markers have been found to be very useful in studying the genetic variability of different species, including *Rosa*. Present studies were undertaken for the identification and analysis of genetic variation within a collection of 4 species and 30 accessions of rose using RAPD analysis technique. The results showed the molecular distinctions among the genotypes when analysed using 25 RAPD primers. Total amplified bands were 146, out of which 110 were polymorphic, with an average of seven bands per primer. Maximum number of bands (10) was produced by primer GLD-20, while GLC-02 produced the minimum number of bands (2). Maximum polymorphism in the present study was obtained by primer GLA-03, GLA-05, GLA-07, GLA-10, GLC-02, GLC-06, GLC-08, GLC-10, OPG-11 and OPE-19 which produced 100% polymorphic bands, while primer GLB-11 produced only 42.85% polymorphic bands. This study demonstrated the potential of RAPD technique for the characterization of genetic variation within the rose germplasm.

Key words: Random amplified polymorphic DNA, polymorphism, rose germplasm, primer.

INTRODUCTION

The rose is the largest and most important ornamental crop in many countries. The genus, Rosa, includes more than 100 species in the temperate and subtropical zones of northern hemisphere and it belongs to the family Rosaceae. According to the system of Rehder (1940), it is divided into four subgenera: *Hulthemia*, *Platyrhodon*, *Hesperhodos* and *Eurosa*, in which the first 3 subgenera include only few species. The subgenus Eurosa comprises 10 sections, and the sections, *Caninae* and *Cinnamomeae*, are the largest and comprised about 50 and 80 species, respectively (Wissemann, 2003). From many of the wild species, a large number of cultivated varieties and hybrids having single or double blossoms that range in colour from white and yellow to many shades of pink and red have been developed. Since many species are highly variable and are hybridized easily, the classification of Rosa is sometimes difficult and the wild type of some modern roses is not always known. The number of modern developed varieties has reached 25,000 with consistent increase in number (Cairns, 2000). This increase in number is going to make the classification to be more complex. In order to improve the exchange of information on varieties, a fast and systematic approach for the selection of varieties essential for direct comparison in the growing test is clearly needed. For this aspect of quality assurance, molecular markers are best suited as they are highly rapid, more economical and have highly discriminating properties within or between species and varieties. After exploiting the potential of multiple molecular markers in several laboratories for identification of rose genotype (Hamrick et al., 1991; Tzuri et al., 1991; Torress et al., 1993; Rajapakse et al., 1993), random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) were found to be the most widely used (Hubbard et al., 1992), as both are useful for identifi-

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Table 1. Names of the four species (A to D) and 30 accessions of *Rosa.*

S/N	Name	S/N	Name
A	*Rosa gruss-an-teplitz* (Surkha)	14	Anjleeq
B	*Rosa bourboniana*	15	Perfect Moment
C	*Rosa centifolia*	16	Mikado
D	*Rosa demescanta*	17	Candia
1	Gold Medal	18	Kardinal
2	Rosy Cheeks	19	Wisky Mac
3	India Cheeks	20	Christian Dior
4	Amalia	21	Oelohama
5	Film	22	Michelle Meilland
6	Gold Pair Gait	23	Maria Shever
7	Shocking Blue	24	Pink Peace
8	Black Rose	25	New Red Rose
9	Multani Rose	26	Pink Cardinal
10	Passion	27	Red Anjleeq
11	Anjleeq Orange Red	28	White Gold Medal
12	Hocus Pocus	29	Nastalgi
13	Perfect	30	Marish Koni Kon

cation, certification and patent protection of roses.

Genetic diversity is one of the important contributors to species survival. Its direct measurement can be an important priority which can further be used for breeding and improvement of these species (Neel and Ellstrand, 2003). In recent years, molecular techniques have been increasingly used in combination with comparative anatomy, morphology and physiology for genetic variability estimation (Weising et al., 2005). The availability of molecular markers facilitates the selection of the desired characteristics in breeding programs and provides the foundation for map-based gene isolation (Koh et al., 1996; Xu et al., 1999). However, identified germplasm is an important component for efficient and effective management of plant genetic resources for any crop.

Indo-Pak subcontinent has always been a sight of attraction for its natural flora and more than 25 rose species have been reported to be growing in this area, which have contributed to the development of modern ornamental roses. The northern areas of Pakistan have many native wild species of roses including *Rosa lacerans*, *R. beggeriana*, *R. brunonii*, *R. canina* and *R. multiflora* with a potential economic value for the area, while several rose species like *R. macrophylla* and *R. webbiana* have been reported in Chitral district of Pakistan (Stewart, 1969). However, earlier rose identification had relied on morphological characters like growth habit, floral morphology, etc (Mohapatra and Rout, 2005). To date, there are only two reports of identification and classification of wild rose species in Pakistan based on morphological and phenotypic traits (Maryum, 2000; Atif Riaz, 2007), since most of the morphological observations are liable to be influenced by variations in soil and environmental conditions. Hence, more observations need to be done for identification of local and exotic rose species and cultivars on genetic basis. In this context, a collection of available rose germplasm in Pakistan (4 species and 30 accessions) was evaluated in the present study to characterize and discriminate these genotypes on a molecular level. The findings will enable the rose breeders to utilize the diverse parents in their effort to develop the improved rose varieties.

MATERIALS AND METHODS

Plant material

The plant material comprised four *Rosa* species and thirty accessions (Table 1) and the leaves of the selected rose germplasm were collected from the experimental area of the department of Floriculture, University of Agriculture, Faisalabad.

Morphological data collection

The plant descriptor for the Rosa species is not well defined and so, based on previous studies, different characteristics including plant height, growth type, the nature of branches, leaf and flower traits were considered important for the morphological studies of the roses. The data were recorded in August to September at full bloom and were analyzed with complete linkage dendrogram for similarities.

DNA extraction

Young leaf tissues of matured plants were used for DNA extraction following Khan et al. (2004). Leaf samples were transferred into zipper plastic bags containing 1.5 ml CTAB, followed by homogenization with a hand roller. After incubation at 65°C for 30 min, the homogenized tissues were transferred into two 1.5 ml eppendorf tubes. Equal volume of chloroform/isoamyl alcohol (24:1) was added and the tubes were inverted 5 to 10 times, followed by centrifugation at 9000 rpm for 10 min (MSB010CX1.5, MSE, UK). The supernatant (about 800 µl) was transferred into a 1.5 ml eppendorf tube, while isopropanol, approximately 700 µl (0.9 volume), was added into the supernatant and mixed by inverting the tube about 10 times. DNA pellet was washed, resuspended in 150 µl of 0.1x TE buffer and quantified by a spectrophotometer (CECIL, CE 2021 2000 Series, Cambridge, UK) at 260 nm. The extracted DNA was electrophoresed on 0.8% agarose gel for quality assessment and verified spectrophotometerically after dilution of 15 ng/µl in ddH_2O.

RAPD analysis

Polymerase chain reaction (PCR) was performed using thermal cycler (Eppendorf AG No. 533300839, Germany) and the conditions were optimized with respect to the concentration of genomic DNA, 10 x PCR buffers, $MgCl_2$, dNTPs, primer and *Taq* DNA polymerase in order to improve the reproducibility of RAPD technique. The PCR was performed in 25 µl reaction volume containing 50 ng of template DNA, 200 µM of each of the four dNTP's, 1x *Taq* polymerase buffer, 1 unit *Taq* polymerase, 2.5 mM $MgCl_2$ and 0.25 µm of the primer. It was performed in thermal cycler

Table 2. Number of amplified bands along with polymorphic bands per primer.

Primer	Amplified bands per primer	Polymorphic bands per primer	% of polymorphic bands
GLA-02	4	3	75
GLA-03	6	6	100
GLA-04	6	5	83.33
GLA-05	7	7	100
GLA-07	7	7	100
GLA-10	6	6	100
GLB-11	7	3	42.85
GLB-15	6	4	66.66
GLC-01	6	3	50
GLC-02	2	2	100
GLC-05	7	5	71.42
GLC-06	3	3	100
GLC-07	7	4	57.14
GLC-08	3	3	100
GLC-09	5	3	60
GLC-10	5	5	100
GLC-11	7	4	57.14
GLC-14	8	4	50
GLC-16	5	3	60
GLC-17	5	4	80
GLC-18	6	4	66.66
-GLD-14	6	3	50
GLD-20	10	7	70
OPG-11	6	6	100
OPE-19	6	6	100

(Eppendorf AG No. 533300839, Germany), using the following cycling program: one cycle of 95°C for 5 min; 40 cycles of 95°C for 1 min; 34°C for 1 min and 72°C for 2 min followed by one cycle of 72°C for 10 min. However, the decamer RAPD primers were custom synthesized by gene link company, UK. Out of 50 random primers, 27 were selected on the basis of their polymorphic nature in the initial study.

Data analysis

The fingerprints were examined under ultra violet transilluminator and photographed using SyneGene Gel Documentation System, while the RAPD bands were counted and designated as present (1) and absent (0). Subsequently, the data were collected and aligned for the construction of cluster analysis and similarity matrix. The cluster analysis of 4 species and 30 rose accessions was constructed by Popgen software (version 1.44) based on Nei's Unweighted Paired Group of Arithmetic Means Averages (UPGMA).

RESULTS

Thirty four rose genotypes generated 146 fragments, using 25 RAPD primers, whereas twenty five primers generated 146 bands, out of which 110 were polymorphic. Considering all the primers and accessions, a total of 75.34% bands were polymorphic, while the rest of the bands (24.66%) were monomorphic. The number of amplified bands produced per primer varied from 2 to 10, with a mean of 5.84 bands per primer. Maximum number of bands (10) was produced by primer GLD-20, while primer GLC-02 produced the minimum number of bands (2). Maximum polymorphism in the present study was revealed by primers GLA-03, GLA-05, GLA-07, GLA-10, GLC-02, GLC-06, GLC-08, GLC-10, OPG-11 and OPE-19 with 100% polymorphic bands, while primer GLC-11 produced only 42.85% polymorphic bands (Table 2).

Multivariate analysis was conducted to generate a similarity matrix using Popgene software, version 1.44 based on Nei's Unweighted Paired Group of Arithmetic Means Averages (UPGMA) to estimate genetic distance and relatedness within species and accessions separately. Assessment on the genetic relationships among thirty four rose genotypes, based on RAPD, can be seen in the dendrogram (Figure 1), which determines the genetic distance among these species and cultivars. From the dendrogram clusters, it is observed that rose genotypes are mainly divided into two groups (that is, A and B). To divide these genotypes in groups, 50%

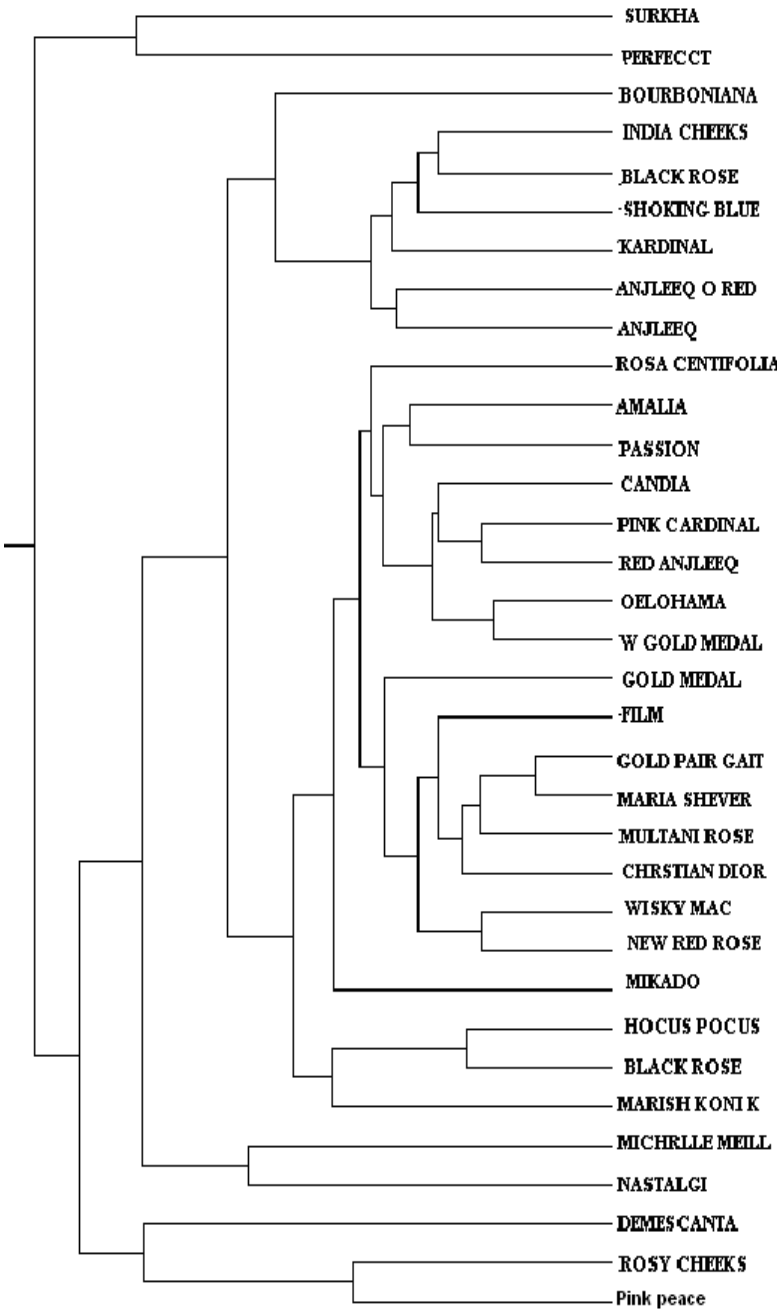

Figure 1. UPGMA dendogram illustrating the genetic relationship among *Rosa* species and cultivars based on Nei and Li's (1979) similarities.

similarity (0.5 similarity coefficient) was taken as the cut-off point. *Rosa gruss-an-Teplitz* (Surkha) and Perfect fall in group A, while *Rosa bourboniana*, *Rosa centifolia*, *Rosa demescanta*, Gold Medal, Rosy Cheeks, Indian Chief, Amalia, Film, Gold Pair Gait, Shocking Blue, Black Rose, Multani Rose, Passion, Anjleeq Orange Red, Hocus Pocus, Anjleeq, Mikado, Candia, Kardinal, Wisky Mac, Christian Dior, OelohamaMichelle Meilland, Maria Shever, Black Rose, New Red Rose, Pink Cardinal, Red Anjleeq, White Gold Medal, Nastalgi and Marish Koni Kon fall in group B.

The data from similarity matrix showed a clear picture of genetic similarities and dissimilarities within rose species and accessions under study. The similarity matrix results indicated that Perfect and Nastalgi were the highest genetic distant lines (that is, 50.86 and 49.14% were genetically similar), whereas Film and Gold Pair Gait were the lowest genetic distant lines (that is, only 5.56 and 94.4% were genetically similar).

DISCUSSION

It is essential to have some basic morphological categorization to assist in sorting and screening of candidate varieties, particularly when the database grows in size. The selected morphological characteristics are useful for this purpose. Experts will have to bear this in mind when using the data coming from different test stations and check across an appropriate range of groups. It is obvious that the influence is the same on the candidate and the reference varieties at one testing station. RAPD markers have the potential for identification of clusters and characterization of genetic variation within the cultivars and species. This is also helpful in rose breeding programs and provides a major input into conservation biology. RAPD produced consistent results with optimized conditions and has the potential to be employed for phylogenetic relationships and taxonomic classification. Like all other techniques, the RAPD-PCR also has limitations, amongst which the complexity of resultant fingerprint patterns is one of them. Another shortcoming of RAPD observed in this study is its low band repeatability and occurrence of pseudo bands. However, it can be improved by applying suitable conditions to remove the impurities in extracted DNA and keeping the amplification conditions stable.

The low genetic diversity in any crop species is a matter of concern which may obstruct further crop improvement. It has been documented that plant improvement is based on the information about the genetic relationships among accessions and within and between species (Thormann et al., 1994). Moreover, plant breeders select a breeding material to breed for elite lines on the basis of genetic relationships among the breeding materials (Hallauer and Miranda, 1988). The tendency to use similar parents extensively in a breeding program has led to a loss of genetic diversity (Fouilloux and Bannerot, 1988).

For the fact that the morphological markers could be influenced by environmental factors, these may not be considered as highly reliable for the taxonomic identification or to measure the genetic relatedness among rose species. On the other hand, molecular markers are independent of these factors and are based on genetic makeup of the individual genotype. Therefore, they are more reliable and should be preferred for further studies to measure genetic diversity in roses.

The findings presented here have implications for rose breeding. The genetic relationships and differences revealed among various rose genotypes under study would be helpful for future breeding programs for rose varietal improvement, as well as selection of genetically distinct parents for germplasm development. However, there is a great need to exploit this flora for improvement in rose varieties, particularly in rose cut flowers industry.

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