International Journal of Plant Production 3 (4), October 2009
ISSN: 1735-6814 (Print), 1735-8043 (Online)
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SHORT COMMUNICATION

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A high genetic diversity revealed between commercial rose cultivars by RAPD-PCR technique

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Received 31 Jan 2009; Accepted after revision 9 August 2009; Published online 17 September 2009

Abstract

Genetic diversity between fifteen commercial cultivars of rose were evaluated, using Ten decamer primers (A-J). All primers detected polymorphism among the cultivars. In total, 126 bands were produced, 73 of which were polymorphic. Primers E and F produced the highest, while primer H produced the lowest number of bands. The percentage of polymorphic bands ranged from 37% to 81% with an average of 63.9%. The average number of polymorphic bands produced was 7.3 per primer. Only the amplified DNA fragments ranging in size between 220 to 3000 bp were used for statistical analyses. Cluster analysis based on the presence or absence of bands was performed by Jaccard's similarity coefficient, based on Unweighted Pair Group Method with Arithmetic Averages (UPGMA). Genetic similarity ranged between 0.12 to 0.53. The dendrogram revealed two main clusters. Each cluster was divided into subgroups. This investigation showed that genetic diversity was relatively considerable among these cultivars. Also, the results propose that RAPD marker is a useful tool for evaluation of genetic diversity and relationships amongst different rose cultivars.

Keywords: Genetic diversity; Molecular Markers; RAPD; *Rosa hybrida*

Introduction

The possible use of *Rosa* germplasm in the future breeding programs depends on the characterisation of the genetic resources and on the study of possible hybridization mechanisms. DNA polymorphism offers direct observations of the plant genotype. One of the techniques used for this purpose is Random Amplified Polymorphic DNA (RAPD) (Manners et al., 2004). RAPD analysis has been used for identification of varieties (Mohapatra and Rout, 2005), phylogenetic relationship (Nair et al., 1999) and conservation and management of genetic resources (Bretting and Widrelechner, 1995). This technique has also been found useful for determining genetic variation (Cubero et al., 1994; Matsumoto and Fukui, 1996; Fredrick et al., 2002). Estimates derived by RAPD are very

similar to those of other methods (AFLP and ISSR) and may be directly comparable (Nybom, 2004). RAPDs have been broadly applied to study taxa of the Rosaceae. These markers proved to be a powerful method for the characterisation of genetic variation in fragrant roses (Prasad et al., 2006; Tabaei et al., 2006; Kiani et al., 2007) and *Rosa canina* (Jurgens et al., 2007). Roses are among the most valuable crops in ornamental horticulture. Despite their commercial significance, little information is available about the inheritance of important agronomic characters. Genetic diversity has been estimated in rose using AFLP (Baydar et al., 2004; Pirseyedi et al., 2005) and microsatellite (Babaei et al., 2007) markers. The aim of this research was to discover distant cultivars to be used in an ongoing rose hybridisation programme in the Research Centre for Plants and Flowers (RCPF), Mahallat, Iran.

Materials and Methods

Plant material

Most plants used in this study were major commercial cultivars and included "Kerem boronzi", "Boukaner yellow", "Nablus white", "Mohammadi", "Limouei", "Chiti", "Columbian", "Golbehi", "Hanaei khareji", "Meshki", "Nastaran", "Nablus yellow", "Narenji" "Mohandesi" and "Banafshe moattar". The plants were given by the Research Centre for Plants and Flowers (RCPF), Mahallat, Iran.

DNA extraction and PCR amplification

The method for DNA extraction was that of Qiang et al., (2004). For PCR analysis, ten 10-mer primers (A-J, Cinnagen Co., Tehran, Iran) were used. PCR was performed in a thermal cycler (Techne, Primus, NWG-Biotech, Germany) in a total volume of 20 µl containing 50 ng of total DNA as the template, 1 µM of primers, 400 µM of deoxynucleotides, 2 µl of 10 X PCR buffer (Cinnagen Co., Tehran, Iran), 1.5 mM $MgCl_2$ and 1 U of Taq polymerase (Cinnagen Co., Tehran, Iran). Conditions applied were as follows: for preheating 3 min at 94 °C, 35 cycles of [1 min at 94 °C for denaturation, 1 min at 35 °C for primers A, C, E, I, and J and 1 min at 40 °C for primers B, D, F, G and H, for annealing, 2 min at 72 °C for extension], followed by an extension for 10 min at 72 °C. The amplified fragments were separated on a 1.5% agarose gel in 1 X TBE buffer (0.089 M Tris-Base, 0.089 M Boric acid and 0.002 M EDTA) at 65 v for 1.5-2 h stained by ethidium bromide (1 µg ml^{-1}) and photographed under UV light in a gel documentation system (UVP, UK).

Results

All decamer primers used on the 15 rose cultivars, generated clear and consistent polymorphic bands (Figure 1). A total of 126 reproducible amplified products were obtained which could be scored with confidence (Table 1).

Although in the RAPD analyses, the intensity and replicability of specific marker bands have not been found very consistent in some studies (e.g. Torres et al., 1993) we found

consistent and reproducible bands in our standard conditions. Figure 2 (a and b) shows the results obtained from primers F and C on the 15 cultivars. In total, 73 polymorphic alleles were produced. Primer F produced 13 bands while primer H produced 3 bands, maximum and minimum number of polymorphic bands, respectively. The highest and the lowest percentage of polymorphic bands were observed for primer F (81%) and primer E (37%). Genetic similarity based on the Jaccard's similarity coefficient in this research was between 0.12-0.53, which is relatively low.

The highest value for genetic similarity was obtained between "Boukaner yellow" and "Mohammadi" cultivars, while the lowest value was obtained between "Nablus white" and "Narenji" cultivars. The average number of polymorphic bands for each primer was 7.3.

Cluster analysis based on presence or absence of bands was accomplished and dendrogram was drawn (Figyre 2). The dendrogram distinguished two major groups. The first group consisted of: "Boukaner yellow", "Mohammadi", "Kereme boronzi", "Limouei", "Chiti", "Mohandesi", "Hanaei khareji", "Meshki", "Nablus white" and "Nastaran" cultivars. The second group consisted of: "Nablus yellow", "Narenji", "Columbian", "Banafshe moattar" and "Golbehi" cultivars. Each group consisted of subgroups. In conclusion, in a hybridization program "Nablus white" and "Narenji" cultivars, could be used as parents, with the aim of producing higher genetic variability.

Discussion

In some research studies using RAPD, it has been shown that domestication, selection and breeding have caused a decrease in genetic variation (McGrath et al., 1999; Fernandez et al., 2001). This phenomenon could have a great impact on several aspects of plants' response to different environmental conditions such as susceptibility to certain diseases. Hence, in order to avoid such problems in future, it seems necessary to estimate genetic diversity between certain plant species and/or cultivars. In this case we chose rose, since it is an important ornamental plant and has found one of the biggest markets of cut flowers internationally.

A relatively simple and easy method for the detection of genetic variation and subsequent decision making for breeding strategies is RAPD-PCR. This technique has been previously employed on different cultivars of rose.

In a similar study Mohapatra and Rout (2005), using 10 RAPD primers could classify 34 rose cultivars into 9 clusters. Despite apparent detectable morphological differences within rose (*Rosa damascena* Mill.) genotypes grown in Turkey, Baydar et al. (2004) observed no genetic variations, employing AFLP and microsatellite markers. They concluded that those rose plants had originated from one genotype and the differences were most probably caused by point mutations. However, in contrast, two other research groups using AFLP, Pirseyedi et al. (2005) and microsatellites Babaei et al. (2007), found significant variations in rose cultivars (*Rosa damascena* Mill.) grown in Iran. As it is clear, our results are in agreement with those of Pirseyedi et al. (2005) Mohapatre and Rout (2005), and Babaei et al. (2007).

Conclusion

These findings support the idea proposed by Debener et al. (1996) that there is a high level of genetic variability, despite the widely accepted opinion of the lack of genetic variability in roses. The results would be used in the breeding programme in the RCPF.

Acknowledgement

Our appreciation is due to Mr. Pirseyedi for his critical guidance in statistic analysis of data.

Table 1. List of primers, their sequences, no. of alleles and size range of products.

Primer	Primer sequence	Size range Of Products (bp)	Polymorphism (%)	No. of polymorphic bands	Total no. Of alleles	Tm (°C)
A	5'-GGTCTCCTA-<G>-3'	280-2000	75	9	12	35
B	5'-CGGAGAGCG-<A>-3'	250-1550	50	6	12	40
C	5'-CCGGCATAG-<A>-3'	270-2400	50	5	10	35
D	5'-TGGGCTCGC-<T>-3'	290-1700	40	6	15	40
E	5'-ACTTGTGCG-<G>-3'	265-3000	37	6	16	35
F	5'-CCCACTGAC-<G>-3'	300-2500	81	13	16	40
G	5'-CTGAGGAGT-<G>-3'	260-1900	75	12	16	40
H	5'-GGTCAACCC-<T>-3'	220-1550	42	3	7	40
I	5'-GCGGGAGAC-<C>-3'	280-1550	45	5	11	35
J	5'-CCTCACCTG-<T>-3'	255-2000	72	8	11	35

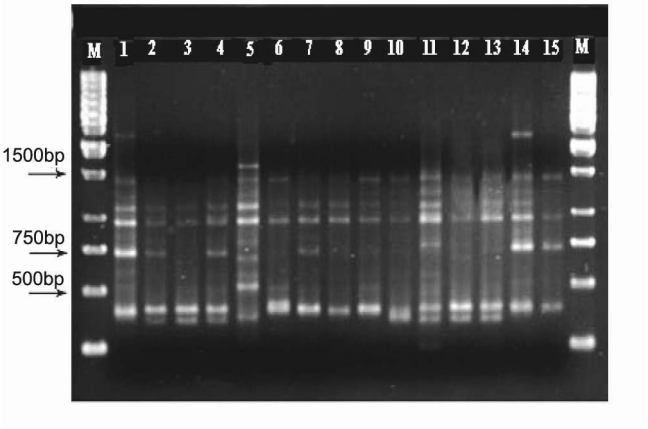

Figure 1. RAPD patterns of fifteen rose cultivars amplified with primer C. Lane M: DNA size marker, Lanes 1 to 15: "Mohammadi", "Banafshe moattar", "Narenji", "Nablus yellow", "Nastaran", "Meshki", "Hanaei khareji", "Golbehi", "Columbian", "Chiti", "Limouei", "Mohandesi", "Nablous white", "Boukaner yellow", and "Kerem boronzi".

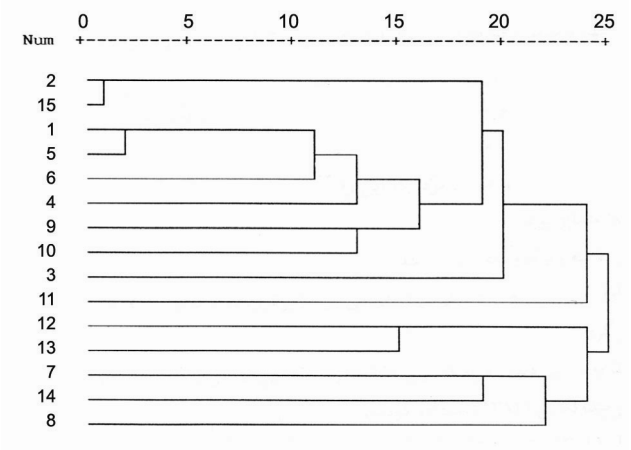

Figure 2. Dendrogram obtained by cluster analysis based on presence/absence matrix. The numbers on the left side correspond to different cultivars, from top "Boukaner yellow", "Mohammadi", "Kerem boronzi", "Limouei", "Chiti", "Mohandesi", "Hanaei khareji", "Meshki", "Nablus white", "Nastaran", "Nablus yellow", Narenji", "Columbian", "Banafshe moattar" and "Golbehi".

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