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RESEARCH ARTICLE

AFLP markers for the assessment of genetic variability in rose (*Rosa gallica* L.) cultivars in Tunisia

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Abstract

Genetic diversity of fourteen Ariana rose (*Rosa gallica* L.) cultivars collected from the north east of Tunisia was investigated using amplified fragment length polymorphism (AFLP). Three AFLP primer combinations produced a total of 533 polymorphic fragments with an average of 177.6 per primer combination. The percentage of polymorphic bands (89.8%), the resolving power (Rp; 99.7) and the PIC (0.51) values showed the efficiency of used primer combinations. The revealed AFLP makers were effective in distinguishing all the cultivars considered; although similarity between cultivars was high (0.53-0.86) demonstrating a narrow genetic background. Cluster (UPGMA) and principal coordinate analyses (PCoA) indicate that the cultivars clustering made independently from the collecting origin. Our results showed that AFLP markers are useful for *Rosa gallica* L. germplasm discrimination as well as for investigation of genetic diversity and variation. The information will facilitate germplasm identification, conservation and new cultivar development.

INTRODUCTION

Information on genetic diversity is essential in optimizing conservation and utilization strategies for plant genetic resources. Several methods to measure genetic distances such as phenotypic descriptors, biochemical and molecular markers have been widely used in crop diversity studies. However, molecular markers have advantages over other kinds; where they show genetic differences on a more detailed level without interference with environmental factors and where they involve techniques that provide fast results detailing genetic diversity (Mondini *et al*., 2009). A large panel of powerful DNA-based methods has been performed and their efficiency has been proven in the description of the polymorphisms within and between species.

Amplified fragment length polymorphism (AFLP), developed by Vos *et al*. (1995), is a rapid and reproducible assay with the ability to generate large numbers of polymorphic genetic loci. This technique is being used extensively for genetic mapping and fingerprinting in plants. It has been used to analyze diversity in many crop species such as *Ficus carica* (Baraket *et al*., 2009), *Gladiolus* (Ranjan *et al*., 2010), *Sorghum bicolor (*Pecina‐Quintero *et al*., 2012), *Miscanthus* (Qin *et al*., 2013) and *Citrus cinensis* (Saddoud Debbabi *et al*., 2014). AFLP allows the retrospective analysis of the consequences of breeding and selection for the production of new lines. The information obtained from AFLP markers can also be used to facilitate the strategic planning of new breeding approaches based on combining and selecting new combinations of genotypes to maximize the rate of line improvement (Vega *et al*., 2006; Xiao *et al*., 2009; Dadras *et al*., 2014).

The genetic diversity among and between *Rosa* species has been investigated in previous studies using morphological, cytogenetical (Bruneau *et al*., 2007) and molecular markers such as isozymes (Kim and Byrne, 1996), random amplified polymorphic DNA (RAPD; Atienza *et al*., 2005; Kiani *et al*., 2008; Mirzaei and Rahmani,

2011; Rai *et al*., 2015), amplified fragment length polymorphism (AFLP; De Cock *et al*., 2008; Koopman *et al*., 2008; Braglia *et al*., 2010) and simple sequence repeats (SSR, Samiel *et al*., 2010; Nadeem *et al*., 2014)

Rosa species were probably introduced to Tunisia in the early beylical era by Spanish Andalusian who fled to Tunisia since 1836 (Ibn Awam, traduced by Clement-Mullet, 1864). The production and utilization of different forms of rose cultivars as ornamental, commercial and industrial crop has been in effect since then. *Rosa gallica* with a very fragrant smell is widely grown in the country and is used for ornament and essential oil extraction. Since his introduction, this species has been acclimated to local environmental conditions and is considered as a native species. Rosa *gallica* is so called 'Ariana rose', related to a department in the North East of the country known by the cultivation of roses. For *ex situ* conservation of the species, some genotypes are cultivated in the rose garden of 'Bir Belhassen' park in the region. A festival called 'Ariana Rose' is organized every year.

In the current study, we report an AFLP-based assessment of genetic variation between 14 *Rosa gallica* L. genotypes in Ariana departement. Ours objectives were to evaluate the usefulness of AFLP for fingerprinting *R. gallica* cultivars and to contribute to the elucidation of genetic relationships between home gardens and *ex situ* rose garden accessions. The information engendered will be of great interest for the management of *in situ* and *ex situ R. gallica* genetic resources in Tunisia.

2. Materials and methods

2.1. Plant material

Fourteen Ariana rose (*Rosa gallica* L.) genotypes were used in this study (Table 1). These include 11 accessions selected from 'Bir Belhassen rose garden' in Ariana Department and 3 accessions collected from home gardens in the same department. A passport data including accession number, collection date, location, collector's names, site name and precise coordinates as obtained by geographical positioning system was assigned for each accession.

2. 2. Morphological description

A brief description of plant materiel was carried out on rose genotypes using morphological parameters related to stem, leaf and flowers according to descriptor lists of UPOV (2010).

2. 3. DNA extraction and AFLP analysis

Genomic DNA from young rose leaves was extracted using CTAB protocol as described by Saghai-Maroof *et al.* (1984). The DNA concentration was estimated spectrophotometrically and its integrity was checked by analytical [1% (w/v)] agarose gel electrophoresis (Sambrook et al., 1989). AFLP analyses were performed following the protocol of Vos *et al.* (1995) except that *Eco*RI selective amplification primers were labeled with VIC and 6-FAM fluorescent dyes at their 5' end (Table 2). The amplified fragments were separated and detected with an ABI PRISM 3130 automatic sequencer (Applied Biosystems) using the POP7 polymer (3130POP7 ^{TM}, Applied Biosystems).

2. 4. Data analysis

Data were collected using GeneScan software version 3.1 (PerkinElmer, Applied Biosystem). The GeneScan samples files were further analysed using GeneMapper version 4.0 (PE, Applied Biosystems). Only clear and unambiguous bands with base pairs ranging from 50 to 500 were coded to a data matrix as present (1) or absent (0) for all the genotypes. Every fragment detected was treated as independent character or allele. The ability of primers to discriminate among cultivars was assessed by calculating the resolving power (Rp) (Prevost and Wilkinson, 1999) which has been reported to correlate between accessions. Evaluation of the Rp was performed according to the formula of Gilbert et al. (1999):

$Rp = \sum Ib$, where Ib=1-[2×|0.5-P|] and P is the proportion of the accessions containing the I band. Moreover, the discriminating of derived markers was made by the assessment of the polymorphism information content (PIC) using the following formula:

$PIC = 1 - \sum_{i=1}^{k} Pi^2$, where k is the total number of alleles detected for a given marker locus and Pi is the frequency of the ith allele in the set of genotypes investigated (Lynch and Walsh, 1998).

Distance analyses and dendogram were also determined using the NTSYSpc version 2.1 for Windows (Rohlf, 2000). Estimates of genetic similarity among all genotypes were calculated according to the formula given by Nei and Li (1979) : $GS_{ij} = 2N_{ij} (N_i+N_j)$, where N$ij$ is the number of bands in common between cultivars i and j, N_i and N_j are the total number of bands in genotype i and j, respectively. The similarity matrix was used to construct a dendrogram by the unweighted pair group method arithmetic averages (UPGMA) procedure (Sokal and Michener, 1958). Principal coordinate analysis (PCoA) was also performed via distance matrix to describe the relationship between accessions using Past program (version 2.0; Hammer *et al*., 2001).

3. Results and discussion

3. 1. Morphological description

The studied rose accessions were characterized by an upright growth habit, a high number of prickles on the stem, reaching 10 to 13 per 10 cm, with a reddish color. The leaves are green and devoid of anthocyanin coloration with a very weak glossiness on the upper side. The terminal leaflet blade has an ovate shape with an acuminate shape of apex (Fig 1 a). Flowers, grouped by 4 to 6 (Fig 1 b), are double with a number of petals greater than 78, a pink color and an irregularly rounded shape (Fig 1 c); the upper part has a flattened convex profile whereas the lower part has a concave profile. The fragrance is very strong.

3. 2. AFLP polymorphism

AFLP analyses are useful and powerful techniques for detecting genetic variations between individuals (Qin *et al*., 2013; Dadras *et al*., 2014). In this study, 3 AFLP primer combinations were used in order to obtain marker information for the inference of genetic relationships among 14 rose genotypes originated from different plantations in Ariana department in Tunisia. We select the most informative primers combinations used by Koopman *et al.* (2008) to generate polymorphic markers for the establishment of phylogenetic relationship between *Rosa* species. The selected primer combinations resulted in 591 different amplification products with 533 polymorphic bands for the 14 individuals. An example of a capillary-AFLP profile using primer combination E-AAG/M-CAT is shown in figure 2.

The average number of polymorphic bands per AFLP primer combination was 177.6. The largest number of polymorphic bands (194) was produced with primer combination E-AAG/M-CAT and the least number of polymorphic bands (158) was detected using primer combination E-AAG/M-CTA (Table 3). The percentage of polymorphic bands (%PB) ranged from 87.7% for E-AAG/M-CTA to 92.3% for E-ACT/M-CAG. Thus, we assume that all tested primers are powerful to detect DNA polymorphisms in rose cultivars. Moreover, estimates of the resolving power (Rp) showed a high rate of collective Rp (99.7), with an average of 33.2. The most informative primer combination for distinguishing the genotypes was E-AAG/M-CAT with the highest Rp value (45.2). The Polymorphism Information Content (PIC) values varied from 0.45 to 0.52 with a mean of 0.51 reflecting a high discriminatory power of the markers for the studied genotypes. The high number of polymorphic bands, the high level of polymorphism within the genotypes suggest that AFLPs are highly discriminatory and powerful markers for classification, fingerprinting and diversity analysis in *Rosa gallica* genotypes as reported in many *Rosa* sections (Koopman *et al*., 2001; Bayder *et al*., 2004; Pirseyedi *et al*., 2005; De Cock *et al*., 2008). In addition, several specific polymorphic fragments were revealed in AFLP patterns of the primer combinations. Then 10 (5.15%) specific fragments were noted for primer combination E-AAG/M-CAT, 12 (6.6%) for E-ACT/M-CAG and 13 (8.2%) for E-AAG/M-CTA. The specific fragments may be cloned and used to generate specific SCAR markers.

3. 3. AFLP-based genetic distance and cluster analysis

Based on the 533 AFLP markers, estimates of genetic similarity exhibited values ranged from 0.53 to 0.86 with an average of 0.67 (Table 4) suggesting that the genotypes studied are characterized by a low divergence at the DNA level. The lowest genetic similarity value of 0.53 has been scored between accessions 25041 and 25040, 25019. However, accession 25010 and 25011 were the closest ones with the highest genetic similarity coefficient of 0.86. All the other ones have different intermediate levels of genetic similarity.

The derived UPGMA dendogram (Fig 3) separated the rose genotypes into several clusters with an average distance between clusters ranging from 0.58 to 1. At the average distance of 0.65, the dendrogram identified 3 main clusters. The largest one (ClI) involved nine accessions from 'Bir Belhassen rose garden'; the similarity coefficient ranged from 0.75 to 0.86 demonstrating a narrow genetic background. Accessions 25039, 25040 and 25041 from home gardens were grouped with accessions 25019 and 25014 from the Rose garden in the second (ClII) and third cluster (ClIII) respectively, indicating a possible common origin.

The principal coordinate analysis (PCoA) was performed according to the two most informative axes PC1 and PC2 that explain more than 68.61% of the variation in the estimates of genetic similarity (Fig 4). The PCoA result was consistent with the topology of the phylogenetic trees constructed with UPGMA and distinguished the groups defined previously in the UPGMA trees. The grouping of the accessions was independent with the collecting origin of the accessions.

Table 1. Analysed rose (*Rosa gallica* L.) accessions

BNGT code	Origin
25010-25020 (11 accessions)	Bir Belhassen rose garden
25039	Soukra
25040	Sidi Saleh
25041	Sidi Thabet

Table 2. DNA sequence of amplified fragment length polymorphism (AFLP) primers and adapters

Type	Name	Sequence
*Eco*RI adapter		5'-CTCGTAGACTGCGTACC-3'
		3'-CATCTGACGCATGGTTAA-5'
*Mse*I adapter		5'-GACGATGAGTCCTGAG-3'
		3'-TACTCAGGACTCAT-5'
*Eco*RI + 1 primer		5'-GACTGCGTACCAATTCA-3'
*Mse*I+ 1 primer		5'-GATGAGTCCTGAGTAAC-3'
*Eco*RI + 3/ *Mse*I + 3 primers	VIC-E1/M1	5'-GACTGCGTACCAATTC*AAG*-3'
		5-GATGAGTCCTGAGTAA*CAT*-3'
	VIC-E1/M2	5'-GACTGCGTACCAATTC*AAG*-3'
		5'-GATGAGTCCTGAGTAA*CTA*-3'
	6-FAM-E2/M3	5'-GACTGCGTACCAATTC*ACT*-3'
		5'-GATGAGTCCTGAGTAA*CAG*-3'

Table 3. Characteristics of AFLP primer combination

Primer combination	Number of alleles		Polymorphism rate (%)	Rp value	PIC value
	Total	Polymorphic			
E-AAG/M-CAT	217	194	89.4	45.2	0.45
E-AAG/M-CTA	180	158	87.7	21.7	0.58
E-ACT/M-CAG	196	181	92.3	32.8	0.52
Total	593	533	--	99.7	--
Average	197.6	177.6	89.8	33.2	0.51

Table 4. Jaccard's coefficient of similarity matrix for 14 Ariana rose (*Rosa gallica* L.) genotypes used in the study

	25020	25011	25012	25039	25040	25015	25017	25018	25010	25014	25013	25019	25041	25016
25020	1.00													
25011	0.78	1.00												
25012	0.66	0.69	1.00											
25039	0.63	0.62	0.56	1.00										
25040	0.60	0.61	0.54	0.75	1.00									
25015	0.76	0.79	0.65	0.62	0.60	1.00								
25017	0.75	0.75	0.63	0.61	0.59	0.76	1.00							
25018	0.80	0.83	0.67	0.63	0.60	0.82	0.77	1.00						
25010	0.77	0.86	0.68	0.63	0.61	0.80	0.74	0.83	1.00					
25014	0.56	0.61	0.66	0.56	0.55	0.57	0.57	0.58	0.62	1.00				
25013	0.78	0.78	0.68	0.64	0.59	0.74	0.74	0.80	0.78	0.63	1.00			
25019	0.61	0.62	0.55	0.74	0.69	0.60	0.60	0.62	0.62	0.56	0.61	1.00		
25041	0.56	0.59	0.64	0.57	0.53	0.54	0.55	0.54	0.57	0.65	0.63	0.53	1.00	
25016	0.68	0.71	0.68	0.58	0.55	0.65	0.65	0.70	0.70	0.64	0.73	0.58	0.63	1.00

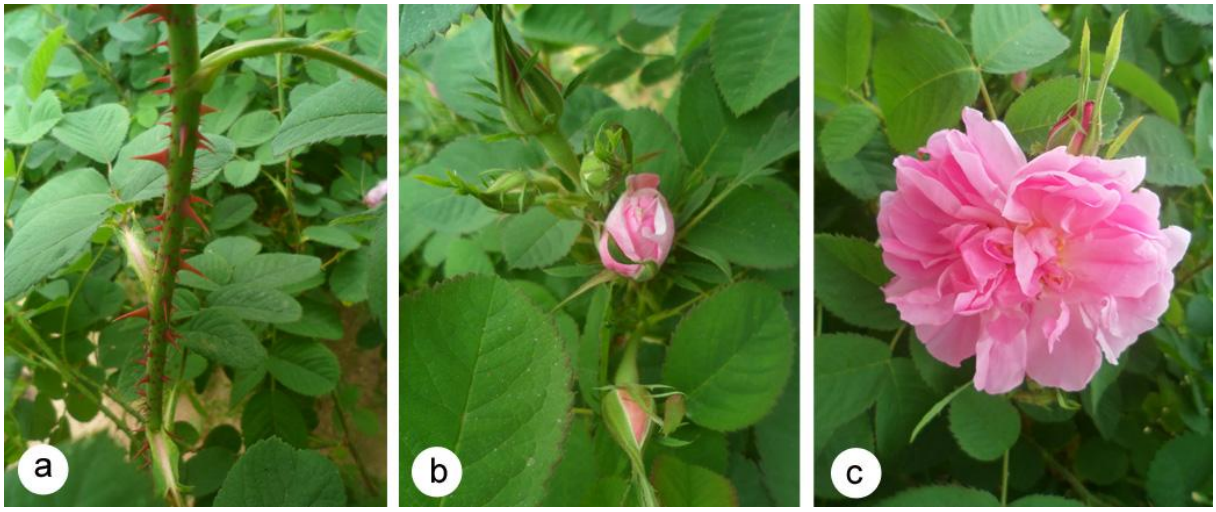

Fig 1. Morphological description of Ariana rose (*Rosa gallica* L.) cultivars used in the study. a. leaf and prickles characteristics. b. inflorescence characteristics. c. flower characteristics.

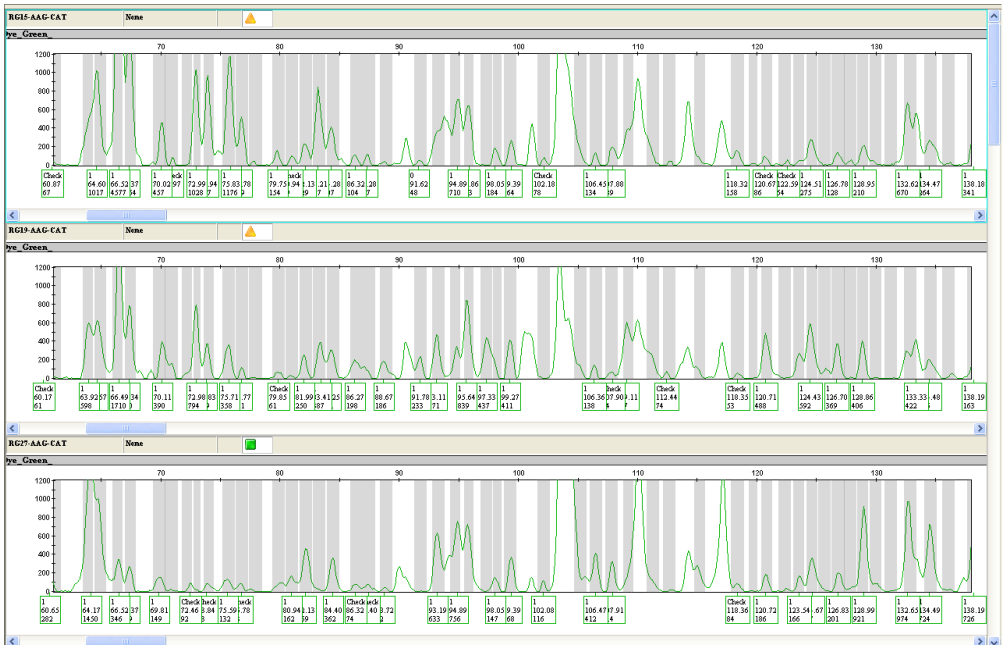

Fig 2. Phenogram image of amplified fragment length polymorphisms detected by the AAG-VIC/CAT primer combination. Only alleles with fluorescence intensities higher than 500 were scored

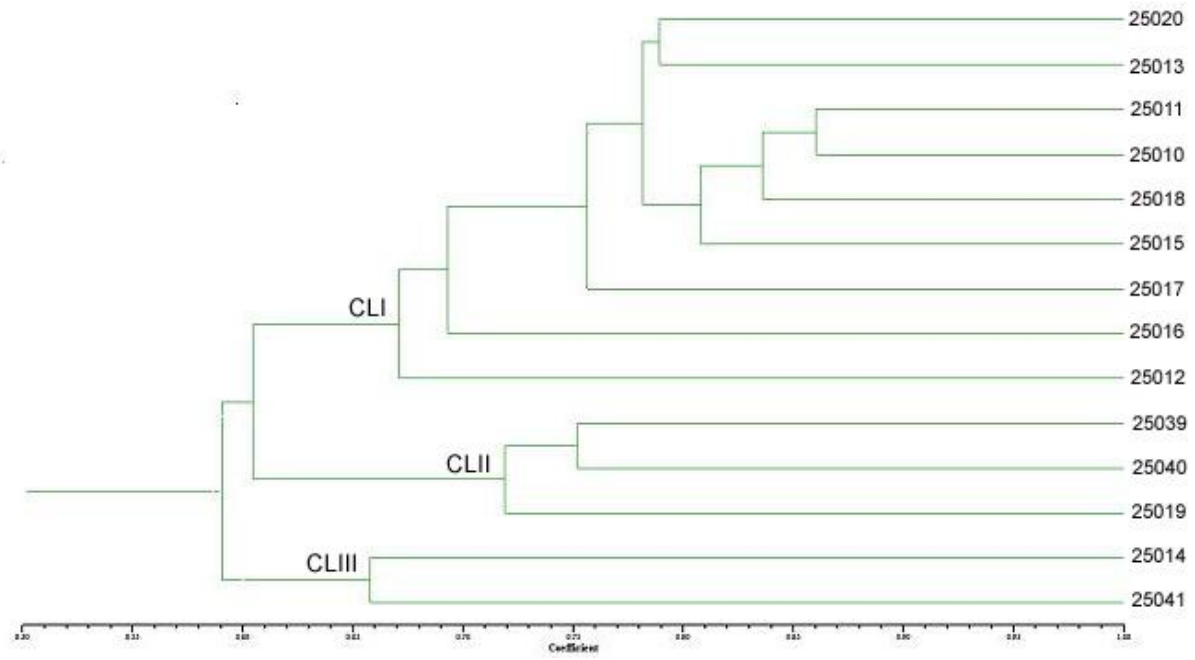

Fig 3. A dendrogram showing the genetic relationships among 14 Ariana rose (*Rosa gallica* L.) cultivars as revealed by 533 AFLP markers

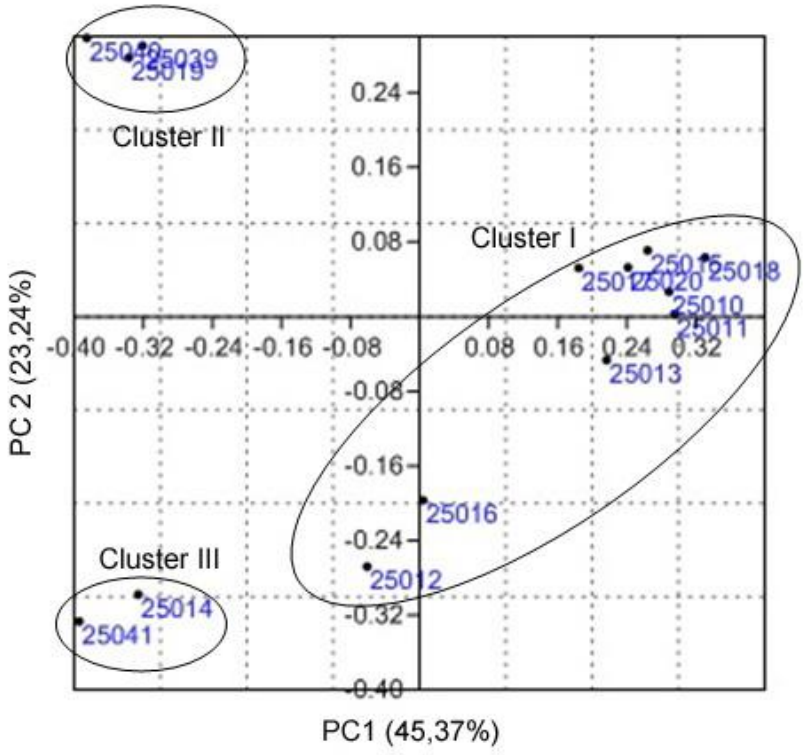

Fig 4. Principal coordinate analysis plot of 14 Ariana rose (*Rosa gallica* L.) cultivars for the first and second principal coordinates

Conclusion

Our results suggest that the AFLP approach is a reliable, rapid and sensitive technique to estimate genetic diversity of Ariana rose (*Rosa gallica* L.) genotypes. AFLP markers were able to group *Rosa gallica* accessions according to their origin and determine genetic similarities between them. Molecular Markers have been found to be effective methods for delineate genetic diversity and structure of populations and can provide effective conservation and management strategies for crop species (Song *et al.*, 2010).

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