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Genetic Relationships of Two *Pulicaria* Species and Identification of Their Putative Hybrids Using Rapd Markers

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Abstract: The objective of this study is to estimate the genetic relationships and dissimilarity among *Pulicaria undulata* (UN) and *Pulicaria crispa* (CR) and their three putative hybrids (N1, N2 and HY) through RAPD markers. Plants were collected from populations at Omdurman South near White Nile, Central Sudan. RAPD analysis was performed using 12 random primers and the results showed that seven primers revealed at least one consistent polymorphic band. The eleven informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships within and between species. A total of 131 amplified fragments were distinguished and 102 polymorphic bands among five candidate species with an average of 8.5 polymorphic markers per primer were obtained. The maximum fragment numbers were obtained using primer B1 with 78.6% polymorphism, while the minimum number was produced using primers; UBC-157 and UBC-104 with 75 and 37.5 % polymorphism, respectively. High dissimilarity was found between hybrids HY and N1 (0.50%) while minimum dissimilarity was found between *P. undulata* and hybrid N1 (0.22%). The study allowed distinguishing the two groups of *Pulicaria* species. The degree of genetic diversity observed between *Pulicaria* species with RAPD markers suggested that this approach was valuable to study the phylogeny of the genus.

Key words: RAPD fingerprinting · Genetic diversity · *Pulicaria* spp

INTRODUCTION

The threat to the genetic diversity present in wild populations may due to some biological and/or environmental factors. The need for preservation of these genetic resources creates an incentive for determination of the genetic variability present within these plant species.

The genus *Pulicaria*, Asteraceae, is comprised of nine species, which are perennial herbs indigenous to northern and central Sudan and have been traditionally used as medicinal plants. Its members are well known for elaborating essential oils. Two *Pulicaria* species, *P. undulata* and *P. crispa*, are currently being most commonly used in Sudan as traditional medicine. *Pulicaria undulata* is rich in phenolic compounds and monoterpene hydrocarbons and comparatively low in sesquiterpene hydrocarbons [1]. The essential oil obtained from steam distillation of aerial parts contain (+)-carvotanacetone, beta-linalool and thymol as major constituents [2]. The oil of *P. undulata* exhibited activity against Gram-positive and Gram-negative bacteria [3]. *Pulicaria crispa* (Forssk.) Benth. and Hook (= *Francoeuria crispa* (Forsk.) Cass.) is pubescent much-branched herb. The presence of sesquiterpene lactones (xantholide, a pseudo-guainolide and a secosesquiterpene lactone) and flavonoids (quercetin, quercetin-3-O-glucoside, quercetin-7-O-glucoside and quercetin-3-methyl ether) have been reported [4,5]. *P. crispa* growing in Saudi Arabia resulted in the isolation of beta-sitosterol, beta-amyrin, choline, quercetin and an unidentified triterpene [6]. The plant is used in folk medicine for the treatment of colds, cough, colic, excessive sweating and as carminative [7].

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Table 1: Polymorphism in five *Pulicaria* species using twelve RAPD markers

Name of primer	Sequence of primer	Total number of bands	Number of polymorphic bands	% of polymorphic
A1	5-AGTCAGCCAC-3	11.0	8.0	72.7
A3	5-GGGTAACGCC-3	10.0	10.0	100.0
B1	5-GTTGCGATCC-3	14.0	11.0	78.6
B20	5-GGACCCTTAC-3	12.0	9.0	75.0
D20	5-ACCCGGTCAC-3	12.0	8.0	66.7
UBC-122	5-GTAGACGAGC-3	10.0	7.0	70.0
UBC-101	5-GCGGCTGGAG-3	12.0	9.0	75.0
UBC-104	5-GGGCAATGAT-3	8.0	6.0	75.0
UBC-155	5-CTGGCGGCTG-3	11.0	9.0	81.8
UBC-157	5-CGTGGGCAGC-3	8.0	3.0	37.5
OPA-15	5-TTCCGAACCC-3	12.0	12.0	100.0
OPA-20	5-GTTGCGATCC-3	11.0	10.0	90.9
Total		131.0	102.0	923.2
Average		10.9	8.5	76.9

MATERIALS AND METHODS

Pulicaria undulata (UN) and *Pulicaria crispa* (Forssk.) species and their three putative hybrids (N1, N2 and HY) were collected from Omdurman Islamic University Campus, AL-Fetihab area, near White Nile, Omdurman South (Khartoum State, Central Sudan).

DNA Extraction and RAPD Analysis: Genomic DNA was extracted from fresh leaf tissue of five candidate species using modified CTAB method [8]. RAPD analysis was performed using eleven 10-mer random primers (Operon Technologies Inc., USA) as shown in Table (1). RAPD a mplification reaction was used in a final volume of 25 µl containing 10X PCR buffer (10 mM Tris-HCl, 50 mM $MgCl_2$), 2 mM dNTPs, 10 mM primer, 50 ng of template DNA and 0.5 U of *Taq* polymerase (Biometra, GmbH, Germany). Reactions were performed in a thermocycler. RAPD-PCR was performed according to Williams *et al.* [9] as one cycle of 94°C for 5 min (denaturation), 40 cycles of {94°C for 1 min, 36°C for 1 min and 72°C for 1 min (annealing)} and a final extension of 7 min at 72°C.

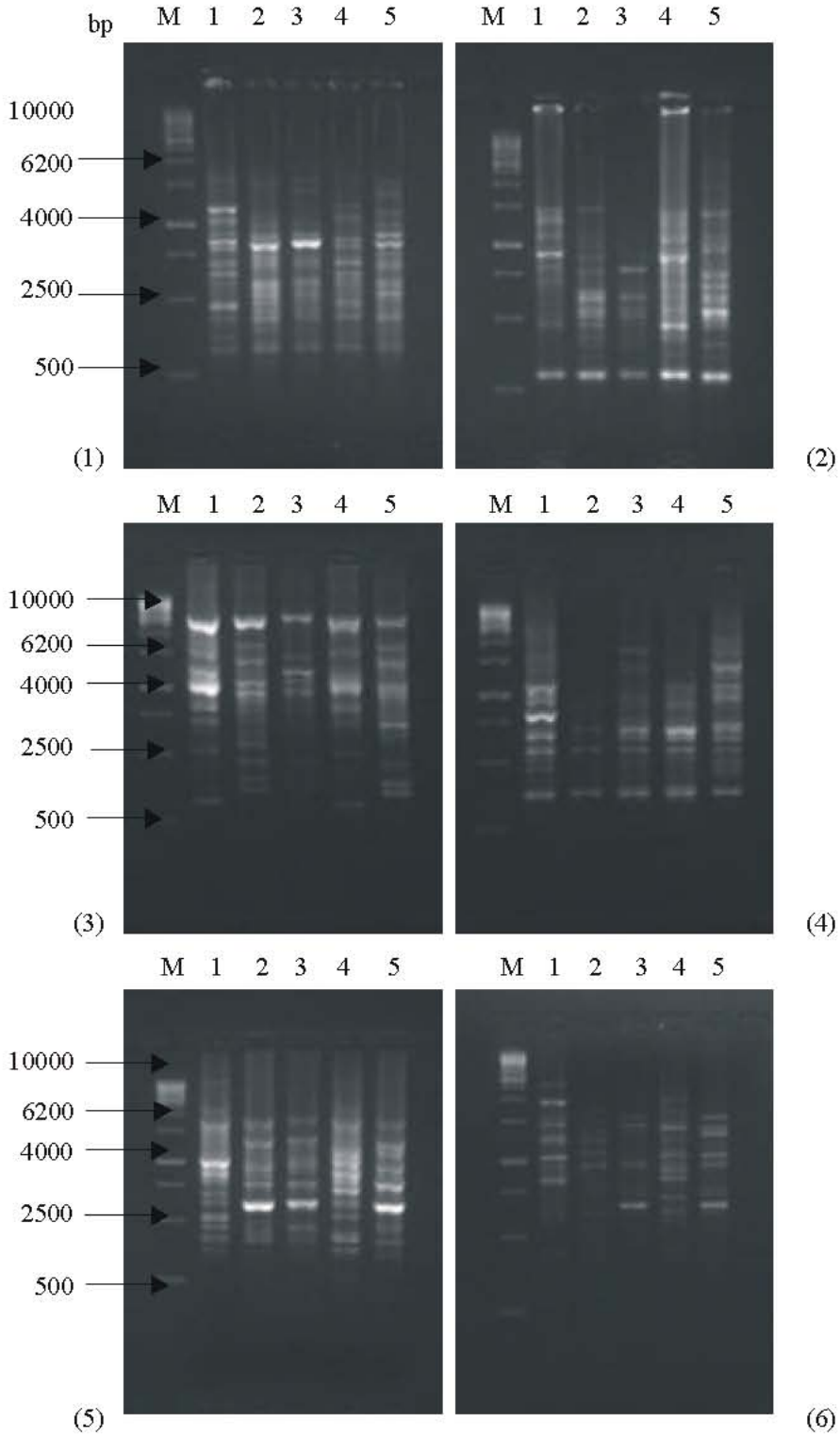
PCR products were analyzed using 1.2% agarose gel electrophoresis and visualized with 10 µg/µl ethidium bromide staining. The sizes of the fragments were estimated based on a DNA ladder of 100 to 1000 bp (Vivantis, Malaysia).

Data Analysis: Bands clearly visible in at least one genotype were scored (1) for present and 0 for absent and entered into a data matrix. The genetic dissimilarity matrix among genotypes was estimated according to Nei and Lei [10]. Coefficient of similarity trees were produced by clustering the similarity data with the unweihted pair group method using STATISTCA- SPSS. The similarity coefficient was used to construct a dendrogram by the unweihted pair group method with arithmetic averages (UPGMA) according to Rohlf [11].

RESULTS

RAPD markers have been used in this study to assessment of genetic relationships among two *Pulicaria* species and their putative hybrids under study. The choice of RAPD technique was motivated by the fact that no DNA sequence information is known about these species. Twelve primers detected enough genetic variation among the five *Pulicaria* species to allow for complete differentiation.

The results indicate that seven primers showing at least one consistent polymorphic band. Eleven primers informative generated reproducible and easily scorable RAPD profiles (Table 1) used to evaluate the degree of polymorphism and genetic relationships within and between all the *Pulicaria ssp.* under study. Total of 131 amplified fragments were distinguished across the selected primers and the statistical analysis showed 102 polymorphic bands among the five *Pulicaria spp.* with an average of 8.5 polymorphic bands per primer (Figure 1). The maximum numbers of fragment bands were produced by the primer B1 (14) with 78.6% polymorphism while the minimum numbers of fragments were produced by the primer UBC-104 (8) with 75 % polymorphism and the primer UBC-157 with 37.5%.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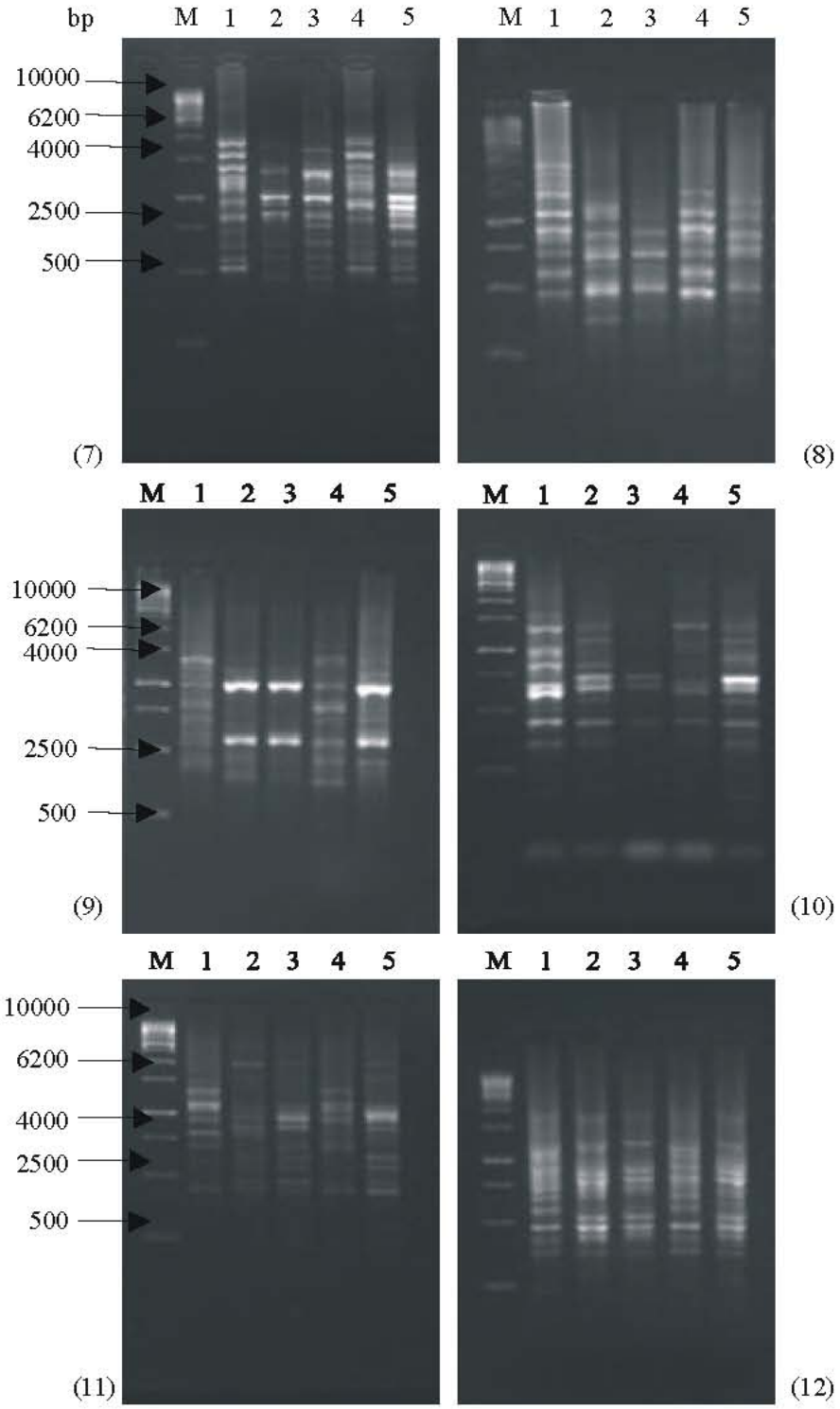

Fig. 1: PAPD profiles of 5 *Pulicaria* species (M = Marker, 1: UN = *Pulicaria undulata*, 2: CR = *Pulicaria crispa*, 3: Hy = hybrid 1, 4: N_1 = hybrid 2, 5: N_2 = hybrid 3) using primer (1) A_1, (2) A_3, (3) B1, (4) B2O, (5) D2O (6) OPA – 15, (7) OPA – 20, (8) UBC- 1 101, UBC - 104, (10) UBC – 122, (11) UBC -155, (12) UBC – 157.

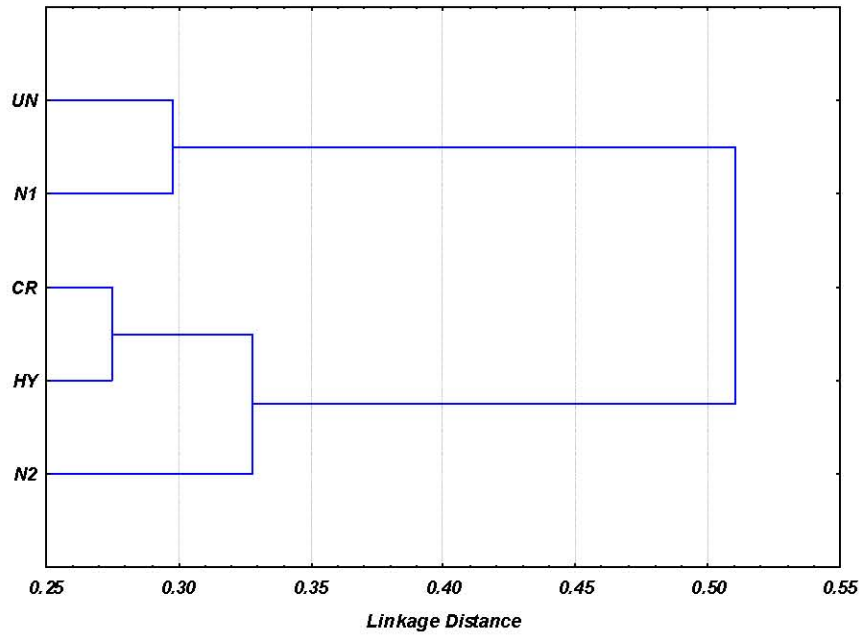

Fig. 2: Dendrogram constructed for five *Pulicaria* species germplasm based on genetic distances

Table 2: Matrix of RAPD dissimilarity among five *Pulicaria* species genotypes based on Nei and Lei coefficients

	UN	CR	HY	N1	N2
UN	0.00				
CR	0.40	0.00			
HY	0.43	0.26	0.00		
N1	0.22	0.44	0.50	0.00	
N2	0.29	0.25	0.28	0.37	0.00

According to the dendrogram, two main groups based on genetic distance were obtained (Figure 2). The first group is indicates UN and N1 which are genetically similar. The second group includes subgroup from CR and HY which are genetically similar and the second subgroup include N2. The cluster analysis showed the relationships between *Pulicaria* species and their hybrids and also proved N1 hybrid came from *P. undulata* and HY hybrid came from *P. crispa*. The dissimilarity matrix obtained after multi variant analysis using Nei and Lei [10] distance is presented in (Table 2). The result of the genetic dissimilarity matrix coefficient indicate that, *P. undulata* had about 0.4 % dissimilarity with *P. crispa* and 0.43 % dissimilarity with hybrid HY. *Pulicaria crispa* had about 0.44% dissimilarity with N1 while HY hybrid had about 0.5 % dissimilarity with hybrid N1. High dissimilarity was observed between hybrids HY and N1. (0.50%) while minimum dissimilarity was observed between *P. undulata* and hybrid N1 was (0.22%).

The genetic variation of *Pulicaria* species and their putative hybrids could broadly be explained as a result of biotic factors, mainly pollination between populations and seed dispersal. An understanding of the genetic diversity responsible for individual species adaptations and responses to their environment (intra specific diversity) is a foundation for understanding almost all ecological and evolutionary processes. The difference found in the dendrogram could be partially explained by different number of loci and there coverage of overall genome in obtaining reliable estimation of genetic relationships among the plant species of Genus *Pulicaria*.

The aerial parts of *Pulicaria crispa*, *P. undulata* and their putative hybrids are more or less similar in morphological traits and difficult to distinguish from each other. Sometimes morphological traits may vary and hence are not reliable for authentication of both medicinal plants and their putative hybrids in dried as well as in fresh state.

RAPD banding patterns have been used for elucidating relationships between subspecies and differentiating a large number of medicinal plants from their close relatives including *Junipers* [12] *Lotus* Campos *et al.* [13] *Solanum astleyl* and *S. bollviense* [14] Chen *et al.* [15] Cheng *et al.* [16] Wolff and Morgan-Richards, [17] Dwivedi *et al.* [18] Forapani *et al.* [19]

Um *et al.* [20] Nieri *et al.* [21] Na *et al.* [22] Zhang *et al.* [23] Ding *et al.* [24] Jayaram and Prasad, [25] Josiah *et al.* [26] Qi *et al.* [27] AL-Gzawi *et al.* [28] Khan *et al.* [29] Makari *et al.* [30] Asili *et al.* [31] Ahlawat *et al.* [32] Khan *et al.*,[33] Ikbal *et al.* [34] Li and Ding [35] Pirkhezri *et al.* [36] Wang *et al.* [37].

CONCLUSION

Benefiting from molecular marker techniques have now become a popular means for identification and authentication of plant and animal species. The study allowed us to distinguish two groups of *Pulicaria* species under study. The first group constitutes the *Pulicaria undulata* and N1 hybrid, the second group constitutes *Pulicaria crispa* and HY hybrid. The analytical tools outlined in this study can be useful for detecting genetic variation and will assist in the conservation and preservation of unique genetic diversity present in plant species collection. Detailed study is needed to understand all the aspect related to relationships. Hence further information is required on patterns of gene flow within and between populations and to assess its impact on population viability.

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