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Original Article

The Genetic and Phytochemical Diversity of Four Populations of Lemongrass (*Cymbopogon olivieri*) from Southeast Iran

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Abstract

Lemongrass (*Cymbopogon olivieri*) is highly cross-pollinated and fragrant herb plant. Because of so worthy essential oil components, it is widely used in cosmetics, food, and medicine industry. The *C. olivieri* leaves of 4 populations were sampled from South and Southeast Iran. Random amplified polymorphic DNA (RAPD) markers used to assess the population structure and genetic variation. In total, 32 polymorphic bands amplified from 11 effective chosen RAPD markers. Cluster analysis using UPGMA method divided the populations into 2 main groups. A high cophenetic correlation coefficient (r = 0.90) was obtained. The pale yellow essential oils were used for GC-MS analysis. Pipertone, carrene, elemol, limonene, benzene, α-pinene, linalool, azulene and calarene were the components with the highest amount found in this study. The high genetic and phytochemical diversity among studied *C. olivieri* population was observed which could be applied in following breeding and gene bank conservation programs.

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Introduction

Plant material

A total of 60 *C. olivieri* genotypes collected from four distribution centers of South and Southeast Iran in 2012. The samples from each region were pooled and kept up separately at -20°C for other laboratory observations. Sampling regions include: Shahdad, Bangood and Zarchin in Kerman province and Baghat in Hormozgan province, all located in Iran. All distribution centers information including: latitude, longitude, and altitude are registered in Table 1.

Table 1. Geographic distribution and continental information of collected *C. olivieri* populations.

Populations	Latitude (N)	Longitude (E)	Altitude (m)
Bangood	25° 17′ 38	55° 31′ 11	2523
Zarchin	26° 29′ 40	55° 31′ 11	2343
Baghat	28° 43′ 17	55° 55′ 17	1161
Shahdad	30° 25′ 3	57° 42′ 24	506

DNA extraction

DNA was extracted from frozen (−20°C) leaves using genomic DNA extraction kit (Qiagen RNeasy Plant Mini Kit, Germany). The eluted genomic DNA solution preserved at -70°C. The concentration of DNA was estimated with a spectrophotometer "Bio Photometer" (Eppendorf, Germany). DNA was diluted up to 100 ng/µl for use in PCR [7].

RAPD-PCR

Fifteen RAPD primers were chosen for preliminary amplification. Eleven RAPD primers (Table 2) with reproducible and score able amplifications characters were selected for next investigations. The primers were synthesized in Isogene Company, Netherlands. PCR reactions were performed in a Thermal Cycler (from Bio-Rad) with a total volume of 25 µl containing 1X PCR reaction buffer, 1.5 mM $MgCl_2$, 0.2 Mm dNTPs, 20 pmol primer, 10 ng template DNA and 1unit of *Taq* DNA polymerase (all provided from Cinagen Company, Tehran, Iran). PCR condition was set at 94°C for 5 min, following 45 cycles of 94°C for 1 min, 30-35°C for 35, 72°C for 1 min, which followed by a final extension of 5 min at 72°C. The samples were run in 1.5% agarose gel in 1X Tris-borate-EDTA (TBE, pH 8.3) buffer.

Gels were run at voltage 90 for 120 min and photographed under UV light using Gel Documentation system. The nucleic acid markers 100 bp-3 kb (Fermentas Company, Germany) was used to compare the amplification product sizes.

RAPD analysis

The RAPD bands were scored for their presence (1) or absence (0) and then transformed into a binary matrix. Polymorphic information content was calculated with total number of bands, number of monomorphic band, and number of polymorphic band. Cluster analysis based on genetic similarities was performed using NTSYS software version 2.02.

Essential oil extraction

Fresh leaves of *C. olivieri* (100 grams in three replication that cut into small pieces (l x1 cm), were subjected to hydro-distillation for 3 h, using Clevenger-type apparatus, according to the method recommended by Guenther (1950) [8]. The extracted essential oils were stored at -20 °C in dark bottles for next experiments.

Table 2. The RAPD primers characterization which utilized for genetic diversity study of four populations of *C. olivieri* in Southeastern Iran.

Count	Oligo Name	Sequence (5'-3')	MW	TM	GC%
1	OPAH-13	TGAGTCCGCA	3028	29.5	60
2	OPJ-10	AAGCCCGAGG	3062	42.5	70
3	OPJ-04	CCGAACACGG	3022	41.2	70
4	OPJ-08	CATACCGTGG	3028	35.3	60
5	OPJ-12	GTCCCGTGGT	3035	42.3	70
6	OPJ-20	AAGCGGCCTC	3013	43.7	70
7	OPJ-7	CCTCTCGACA	2948	36.5	60
8	OPJ-11	ACTCCTGCGA	2988	40.1	60
9	OPJ-16	CTGCTTAGGG	3059	34.5	60
10	OPAJ-19	ACAGTGGCCT	3028	40.5	60
11	OPAJ-13	CAGCCGTTCC	2964	41.2	70

GC-mass spectrophotometry

GC analyses were performed, using a Shimadzu Qp5050 gas chromatograph equipped with a DB-1 fused silica capillary column (30 m · 320 lm i.d., film thickness 0.25 µm, J and W Scientific, Folsom, CA, USA). GC was connected with a mass spectrometer (Shimadzu Crop., Kyoto, Japan). Oven temperature was held at 50°C for 2 min and then programed to 250°C at a rate of 5°C per min. Detector (FID) temperature was 280°C and injector temperature was 250°C; Helium was used as carrier gas with a linear velocity of 1.1 ml/min spilt ratio 1:20, ionization energy 70 eV, and mass range 35–390 a.m.u. The percentages of compounds were calculated by the area normalization method, without considering response factors.

Results

Rapid analysis

Eleven of fifteen RAPD primers could amplify reproducible and scorablebands for screening of *C. olivieri* genetic diversity. A total of 42 bands were amplified by entire utilized primers which 32 of them were polymorphic bands (Table 3). OPJ-12 primer amplified the highest scorable band (8) which 6 of them were polymorphic. The primers of OPJ-04, OPJ-8, OPAJ-13, and OPJ-7 amplified just polymorphic bands (Table 3). The size of amplified fragments was between 100-800 bp.

Genetic identity and genetic distance

To further elucidate the gene differentiation between populations, Nei's original measure of genetic identity (IN) and genetic distances (D) were evaluated (Table 4). IN ranged from 0.3181 to 0.5909 and D ranged from 0.4091 to 0.6819, in which the Baghat and the Shahdad populationshad the highest genetic identity (IN = 0.5909) and closest in the genetic distance (D = 0.4091). Bangood population had the lowest in genetic identity (IN = 0.3181) and the furthest in genetic distance (D = 0.6819) with Shahdad and Baghat populations.

Table 3. The RAPD primers name, band size, number of polymorphic and monomorphic bands, and polymorphism rate (%) which utilized for genetic diversity study of four population of *C. olivieri* in Southeastern Iran.

Primer	Band size	Polymorphic bands	Monomorphic bands	Total bands Polymorphism rate %
OPAH-13	100-300	3	2	5 60 %
OPJ-04	600	1	0	1 100 %
OPJ-08	300	1	0	1 100 %
OPJ-10	500-700	2	0	2 100 %
OPJ-12	300-700	6	2	8 75 %
OPJ-20	500-800	3	1	4 75 %
OPAJ-19	400-800	3	1	4 75 %
OPAJ-13	100-300	5	0	5 100 %
OPJ-7	100-800	4	0	4 100 %
OPJ-11	100-800	2	2	4 50 %
OPJ-16	200-800	2	2	4 50 %

Table 4. Nei's original measures of genetic identity and genetic distance among *C. olivieri* populations in Southeastern Iran with RAPD markers.

Population ID	Baghat	Shahdad	Zarchin	Bangood
Baghat	****	0.5909	0.4318	0.3181
Shahdad	0.4091	****	0.3864	0.3181
Zarchin	0.5682	0.6136	****	0.5227
Bangood	0.6819	0.6819	0.4773	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Clustering analysis of 4 C. olivieri populations

Cluster analysis revealed 2 main clusters. Shahdad and Baghat populations placed in cluster 1 with 0.5909 similarity Fig. 1). Zarchin and Bangood populations placed in cluster 2 with 0.5227 of similarity (Fig. 1). Both clusters connect to each other in similarity coefficient of 0.3864 (Fig. 1). The cophenetic correlation coefficient indicated high correlation between the similarity matrix and the cophenetic matrix obtained from the UPGMA dendrogram (r = 0.90), indicating a good representation of clustering.

GC-MS analysis

A pale yellow essential oil (0.9-1 %) was obtained from fresh leaves of Southeastern lemongrass plants. These results are in consent with previous reports which remarked the essential oil content should average 0.50–1% in normal condition [9-10].

The GC-MS results determined thatthe essential oil of *C. olivieri* population of Zarchin contained 11 components (Fig. 2) and the components with the highest amount were pipertone, carrene, elemol, limonene, benzene, and cala-

rene containing 58.01, 12.86, 8.76, 3.35, 2.71, and 2.07%, respectively (Table 5). The analytic analysis of Bangood *C. olivieri* population essential oil determined 9 components which the components of piperitone, elemol, α-pinene, linalool, benzene, and limonene had the highest amounts containing 30.95, 6.90, 6.54, 5.73, 1.49, and 1.46 % respectively (Table 5). In Shahdad *C. olivieri* population essential oil, the 6 components were determined in which the components of careen, terpineol, piperitone, and limonene had the highest amounts with having 16.57, 14.937, 13.197, and 4.17%, respectively (Table 5). The GC-MS results determined that the essential oil of *C. olivieri* population of Baghat contained 9 components and the components of pipertone, terpinene, azulene, camphene, and limonene had the highest amounts with having 25.78, 8.44, 3.75, 3.01 and 2 %, respectively (Table 5).

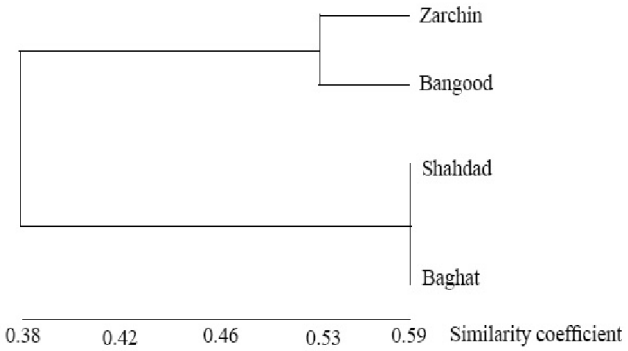

Figure 1. The dendrogram of 4 *C. olivieri* populations by RAPD analysis based on unweighted pair-group method with arithmetic average (UPGMA).

Discussion

The RAPD analysis placed 4 populations of *C. olivieri* from Southeast Iran in 2 main groups. The 32 of 42 obtained loci from utilized RAPD primer showed polymorphic bands. Therefore, dissimilarity between populations remarked well. Although, population from almost equal geographic characteristics and atmosphere with close distance, placed in the same cluster (Zarchin and Bangood) which can be because of wild type plants adaptation in a special environment during long-term natural selection pressure. But, some populations despite unequal geographic characteristics and atmosphere with long distance placed the same cluster (Shadad and Baghat) which can be associated with migration or genetic exchanges current. This result is in agreement with the results of Adhikari *et al*., (2013) [11] which obtained 52 ploymorphic bands from a total of 64 bands by implementing 12 RAPD markers in 10 elite Indian cultivars of Cymbopogon. These results proved that RAPD markers are an efficient marker system in lemongrass plants with regard to detection of polymorphism. Also, Ganjewala (2008) [12] clustered 3 selected cultivars, two lemon scented OD-19 and Krishna and one rose scented GRL-1 of East Indian lemongrass (Cymbopogon flexuosus (Nees ex Steud)) with use of 19 OPJ 10 mer RAPD markers. The cluster analysis showed that cv. GRL-1 was very closely related with cv. OD-19 while Krishna slightly distant from cv. OD-19 than GRL-1.

Table 5. GC-MS analysis of *C. olivieri* essential oil from 4 populations of Southeastern Iran.

Compound	RT	Bangood	Zarchin	Shahdad	Baghat
Benzen %	9.341	1.42	2.71	1.74	1.03
Carrene %	10.525	ND	12.86	16.57	ND
Limonene	11.502	1.46	3.35	4.17	2
Alph-Pinene %	11.899	6.54	1.65	ND	1.21
Terpinene %	14.682	ND	1.70	ND	8.44
Terpineol %	16.898	1.18	1.82	14.937	ND
Piperiton %	19.109	30.95	58.01	13.197	25.78
Elemol %	25.560	6.90	8.76	ND	ND
Naphthalenmethanol %	27.833	1.05	0.88	ND	1.55
Valencenev%	28.531	ND	2.05	ND	ND
Calarene %	28.941	ND	2.07	ND	ND
Camphene %	8.707	1.42	ND	ND	3.01
Linolool %	14.068	5.73	ND	ND	ND
Azulene %	29.067	ND	ND	1.44	3.75
Borneol %	16.136	ND	ND	ND	1.55

ND: not determined.

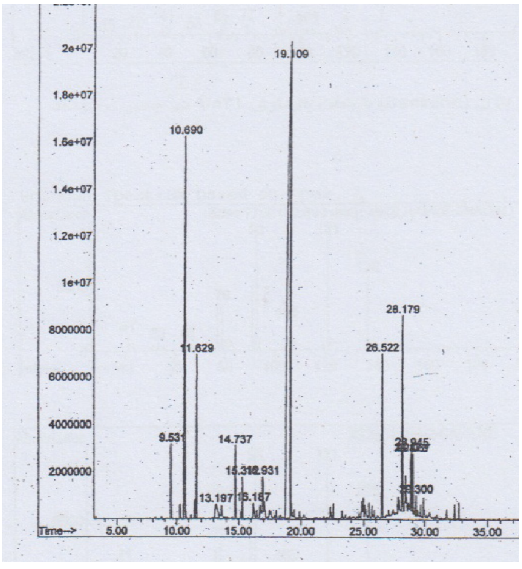

Figure 2. Gas chromatogram of *C. oliveri* essential oil (Zarchin population).

Nowadays, essential oils as the major therapeutic, flavor and fragrance agents to treat several diseases, in cosmetics and food industries have become an integral part of everyday life. The GC-MS analysis indicated that the major component of essential oil in all populations was piperitone. It is a natural monoterpene ketone and used as the principal al raw material for the production of synthetic menthol and thymol. Ketoh *et al*., (2006) [13] study indicated insecticidal activity of piperitone against *Callosobruchus maculatus*. Also, Guzel *et al*., (2017) [14] reported antifeedant activity against *Spodoptera littoralis* (Boisd). Naphthalene and azulene are other organic compounds of Southeast Iran *C. olivieri* essential oil. Azulene is an isomer of naphthalene, whereas, naphthalene is colourless and azulene is dark blue. Azulene used mainly as a blue coloring agent in cosmetics [15]. However, it has also been shown to have anti-inflammatory and antioxidant properties. Limonene, as other component of studied *C. olivieri*, is a colorless liquid hydrocarbon classified as a cyclic terpene. Limonene commonly used in cosmetic products including: fragrance in perfumery, aftershave lotions, bath products, and fragrance. Also, d-limonene is used in food manufacturing and some medicines [16].

Conclusion

In conclusion, it should be mentioned that this research denoted high genetic and phytochemical diversity among studied *C. olivieri* population. It could be applied in following breeding and gene bank conservation programs to improve their appropriate characteristics which are utilized in industry and medicine.

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