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Analysis of Genetic Diversity among Populations of Common Reed (*Phragmites australis*) in Iran

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ABSTRACT

The common reed (*Phragmites australis*) is a perennial weed with the largest geographical distribution of any flowering plant in the world. In this study, 39 populations of common reed were collected and random amplified polymorphic DNA (RAPD) markers were used to characterize the genetic diversity among these populations. The 16 primers used in this study, amplified 149 scorable RAPD bands among which 123 were polymorphic (82%). Genetic similarity coefficients ranged from 0.44 to 0.84, with an average of 0.60. Results showed that RAPD markers are suitable for the evaluation of genetic diversity of common reed. The results support the clonal nature of *P.australis* with its growth and spread primarily by vegetative propagation.

Key words: common reed, *Phragmites australis*, RAPD markers, genetic diversity.

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چکیده:

نی از علف های هرز چندساله است که وسیع ترین پراکنش را در بین گیاهان گلدار دنیا دارد. ۳۹ جمعیت نی در ایران جمع آوری شد و تنوع ژنتیکی بین آنها با استفاده از نشانگرهای RAPD بررسی شد. ۱۶ پرایمر ۱۴۹ باند تولید کردند که ۸۲٪ آن یعنی ۱۲۳ باند، پلی مورفیک بود. ضرایب شباهت ژنتیکی بین جمعیت ها از ۰/۴۴تا ۰/۸۴ متغیر و متوسط شباهت ژنتیکی ۰/۶۰ بود. نتایج آزمایش نشان داد که نشانگرهای RAPD برای بررسی تنوع ژنتیکی نی، مناسب بودند. هچنین شباهت ژنتیکی بین جمعیت ها اهمیت تکثیر رویشی را در این علف هرز نشان می دهد.

INTRODUCTION

The common reed [(*Phragmites australis*) (Cav.) Trin. ex steud,] is a perennial grass with the largest geographical distribution of any flowering plant in the world (Clevering & Lissner, 1999). *Common reed*enlarges its populations generally by clonal growth through rhizomes (Dong, 1996) and therefore is a typical species of plural clonal plants. In the last few years, the field of molecular biology has provided new tools for the population structure. In the late 1960s and 1975, clonal diversity and evolutionary processes in wetland species were studied for the first time, using allozyme polymorphisms on *Typha* and *Spartina* species (McNaughton, 1975; Silander, 1985, Raybould *et al*., 1991). From the 1980s, new perspectives in how to study evolutionary processes and population dynamics in *Common reed*became available with the development of molecular markers (Jackson *et al*., 1985; deKroon & Van Groenendael, 1997). However, still our knowledge of the underlying evolutionary processes determining clonal diversity is limited. While morphological and physiological characteristics are often influenced by the environment, genetic markers may present the most reliable method of distinguishing different population types, since they are not affected by environmental factors (Saltonstall, 2003 a).

Different molecular techniques have been employed to detect clonal diversity in *Common reed*such as allozymes (Hauber *et al*., 1991; Clevering & Lissner, 1999; Clevering *et al*., 2001; Pellegrin & Hauber; 1999), restriction fragment length polymorphisms (RFLPs) (Koppitz *et al*., 1997; Saltonstall, 2003a), random

amplified polymorphic DNAs **(**RAPDs**)** (Neuhaus *et al*., 1993; Kuhl *et al*., 1999; Keller, 2000; Koppitz & Kuhl, 2000; Curn *et al*., 2007), amplified fragment length polymorphisms (AFLPs) (Lambertini *et al*., 2006), microsatellite (SSR) analysis, and chloroplast DNA sequencing (Saltonstall, 2002, 2003 b). The random amplified polymorphic DNA technique, developed in 1990 (Williams *et al*., 1990) has been recognized as a more accessible technique, as it has a relatively low cost and requires very small quantities of genomic DNA (Ragot & Hoisington, 1993; Russell *et al*., 1997). It is a fairly simple method which many studies have used for clarification of nomenclature (Keil & Griffin, 1994), identification of herbarium accessions (Khadari *et al*., 1995), or elucidation of genetic relationships (Russell *et al*.,1997).

Published studies on population genetic variation of *P.australis* have focused on geographically localized populations in Europe and USA (Djerbrouni, 1992; Kuhl & Neuchaus, 1993; Neuhaus *et al*., 1993; Zeidler *et al*., 1994; Mckee & Richards, 1996; Koppitz *et al*., 1997; Pellegrin & Hauber, 1999; Bastlova *et al*., 2004; Bastlova *et al*., 2006; Hansen *et al*., 2007) and not much study has been done in Iran. This study investigated the genetic diversity among populations of *Common reed*in Iran using RAPD markers.

MATERIALS AND METHODS

Plant Material and DNA Extraction

The rhizomes of 39 populations of common reed were collected from different regions of Iran during November 2005 (Figure. 1). Abbreviations were used to make it easy to work out different populations of this species (Table 1).

Figure 1**.** The geographic origin of the 39 populations of *Common reed*used in this study.

*Common reed*leaf samples were taken and stored at -20^{o}C until preparation. DNA for PCR assay was isolated according to the CTAB method of Rogers and Bendich (1985) and stored in 1x TE solution at +4°C.

Table 1. Collection codes and localities of sampled populations of *P.australis*.

No.	Origin site	Province	Codes	Latitude N°	Longitude E °	Altitude (m)
1	Moghan	Ardebil	A-MO1	39° 33′	47° 46′	170
2	Moghan	Ardebil	A-MO2	39° 30′	47° 44′	165
3	Moghan	Ardebil	A-MO3	39° 31′	48° 01′	172
4	Moghan	Ardebil	A-MO4	39° 33′	48° 03′	174
5	Moghan	Ardebil	A-MO5	38° 15′	47° 54′	168
6	Moghan	Ardebil	A-MO6	38° 50′	47° 04′	171
7	Mesgaran	Khorasan	KO-ME	36° 47′	55° 43′	-
8	Mashhad	Khorasan	KO-MA	36° 20′	55° 06′	-
9	Nazarie	Khorasan	KO-NA	36° 08′	55° 25′	-
10	Dezfool	Khoozestan	KZ-D1	32° 15′	48° 26′	82
11	Dezfool	Khoozestan	KZ-D2	32° 14′	48° 27′	83
12	Ojirob	Khoozestan	KZ-OJ	32° 12′	48° 29′	88
13	Motahhari-shahrak	Khoozestan	KZ-MO	32° 16′	48° 22′	87
14	Shoosh	Khoozestan	KZ-S1	32° 54′	47° 26′	-
15	Shoosh	Khoozestan	KZ-S2	32° 45′	46° 35′	-
16	Sarabeyavari	Kermanshah	KE-S1	34° 29′	46° 56′	1072
17	Sarabeyavari	Kermanshah	KE-S2	34° 39′	46° 19′	1073
18	Bisotoon	Kermanshah	KE-BI	34° 22′	47° 26′	1305
19	Mahidasht	Kermanshah	KE-MA	34° 19′	47° 52′	1410
20	Shahre-Ray	Tehran	T-SHR	35° 33′	51° 22′	-
21	Sahrake-Sinamayi	Tehran	T-SHS	35° 27′	51° 13′	-
22	Varamin-Goltape	Tehran	T-VGO	35° 03′	51° 30′	-
23	Varamin-Gharchek	Tehran	T-VG1	35° 09′	51° 36′	-
24	Varamin-Gharchek	Tehran	T-VG2	35° 13′	51° 40′	-
25	Varamin-Gharchek	Tehran	T-VG3	35° 20′	51° 47′	-
26	Dolat Abad	Tehran	T-DO1	35° 46′	51° 10′	1289
27	Dolat Abad	Tehran	T-DO2	35° 40′	51° 02′	1289
28	Mohammad Shahr	Tehran	T-MO1	35° 50′	51° 06′	1296
29	Mohammad Shahr	Tehran	T-MO2	35° 57′	51° 14′	1298
30	Beheshtmasoome	Qom	Q-BE1	34° 30′	51° 16′	-

31	Beheshtmasoome	Qom	Q-BE2	34° 39′	51° 18′	-
32	Beheshtmasoome	Qom	Q-BE3	34° 59′	51° 30′	-
33	Sari	Mazandaran	MA-S1	36° 37′	52° 56′	6
34	Sari	Mazandaran	MA-S2	36° 29′	53° 04′	7
35	Gorgan	Golestan	G-GR1	36° 07′	54° 16′	4
36	Gonbad	Golestan	G-GO1	37° 10′	54° 43′	5-
37	Gonbad	Golestan	G-GO2	37° 47′	54° 51′	3-
38	Gorgan	Golestan	G-GR2	36° 30′	54° 05′	2
39	Gorgan	Golestan	G-GR3	36° 51′	54° 28′	5

RAPD assay

The RAPD analysis was performed using a set for 16 random primers (Table 2) of all common reed populations. Polymerase chain reactions (PCR) were carried out in a 25 µl reaction mixture containing 1.9 m*M* $MgCl_2$, 0.5 µl primer, 1.25 x PCR Buffer, 0.2 m*M* of each dNTPs, approximately 100 ng genomic DNA and 1 U Taq DNA polymerase. Amplifications were performed in a thermocycler according to a program that included an initial denaturing step of 1 min at 92° C then 45 repeats of 1 min at 35° C, 2 min at 72° C, and a final of 72° C for 4 min. Final step was followed by a holding temperature of 4° C. Amplification products were separated in 1.5% agarose gels run in 1 × TAE buffer and detected by staining with ethidium bromide. RAPD fingerprints were amplified repeatedly (the same results were obtained in three independent PCR experiments). The clear and distinct banding pattern indicates that this method is suitable for studies of common reed.

Data Analysis

For each population data were scored as 1 for the presence and 0 for the absence of a DNA band. The data matrix was analyzed by ("given to" or "processed by") NTSYS (Rohlf, 1998) and analyzed using the qualitative routine to generate Dice similarity index as in Nei & Li (1979). Dendrogram showing genetic relationships of the 12 landraces structured using the unweighted pair-group method with arithmetic averages (UPGMA) (Koppitz, 1999).

Table 2. RAPD primers used along with their sequence of nucleotides and the percentage of polymorphic bands produced by each primer.

Primer code	Nucleotide sequence 3´ → 5	Number of total bands	Percentof polymorphic bands
UBC 1	CCT GGG CTT C	15	92
UBC 3	CCT GGG CTT A	8	87.5
UBC5	CCT GGG TTC C	9	80
UBC9	CCT GCG CTT A	9	80
UBC 13	CCT GGG TGG A	9	73.3
UBC16	GGT GGC GGG A	8	88
UBC64	GAG GGC GGG A	9	85
UBC 66	GAG GGC GTG A	10	75.6
UBC76	GAG CAC CAG T	8	90
UBC77	GAG CAC CAG G	9	80
UBC82	GGG CCC GAG G	8	75
UBC 84	GGG CGC GAG T	10	90
UBC89	GGG GGC TTG G	8	83.3
UBC95	GGG GGG TTG G	10	80.9
UBC96	GGC GGC ATG G	10	86.9
UBC100	ATC GGG TCC G	9	83.3

RESULTS AND DISCUSSION

A total of 149 bands were screened (average of 9.31 bands per primer) among which 123 were polymorphic. The number of bands per primer varied from 8 to 15 with an average of 9.31. The average proportion of polymorphic markers across primers was 83.1%, ranging between 73.3 % (UBC13) and 92% (UBC1) (Table 2). Estimates of genetic similarity of RAPD, based on 123.1 polymorphic markers between 39 populations of common reed ranged from 0.44 to 0.84 with an average of 0.60 (Figure. 2).

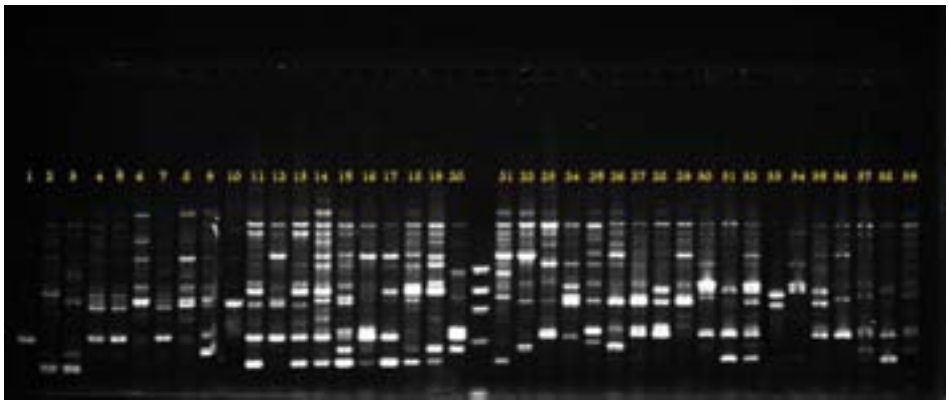

Figure 2. PCR fingerprints of *P.australis* DNA of 39 populations primed with UBC66.

Diggle *et al*. (1998), Ellstrand & Roose (1987), Widen *et al*. (1994), and Khudamrongsawart *et al*. (2004) reviewed literature regarding genetic diversity in clonal plant population and found substantial amounts of diversity in most plant species. European studies of common reed, examining the genetic variation and population genetic structure, seem to support the summary on clonal variation by Loveless and Hamrick (1984) of high levels of genetic divergence among populations (Kuhl & Neuhaus, 1993; Zeidler *et al.*, 1994; Koppitz *et al*., 1997) and either homogenous (Koppitz *et al*., 1997) or heterogenous populations (McKee & Richards, 1996) or a combination of the two (Kuhl & Neuhaus, 1993; Zeidler *et al*., 1994).

Cluster analysis resulted in the grouping of 39 populations into 4 main clusters in 0.62 distance unit (Figure 3). First cluster had two subdivisions. First subdivision contained six populations form Ardebil (A-MO1, A-MO2, A-MO3, A-MO4, A-MO5 and A-MO6), three populations from Khorasan (KO-MA, KO-ME and KO-NA) and four populations form Kermanshah (KE-S1, KE-S2, KE-BI and KE-MA). Second subdivision contained six populations from Khoozestan (KZ-SH, KZ-OJ, KZ-D1, KZ-D2, KZ-A1 and KZ-A2), one population from Mazandaran (MA-S2) and 10 populations from Tehran (T-SHR, T-SHS, T-VGO, T-VG1, T-VG2, T-VG3, T-DO1, T-DO2, T-MO1 and T-MO2). Second cluster

included populations from Qom (Q-BE1, Q-BE2 and Q-BE3). Five populations from Golestan (G-GR1, G-GR2, G-GR3, G-GO1 and G-GO2) form the third cluster. The fourth cluster contained only one population from Mazandaran (MA-S1).

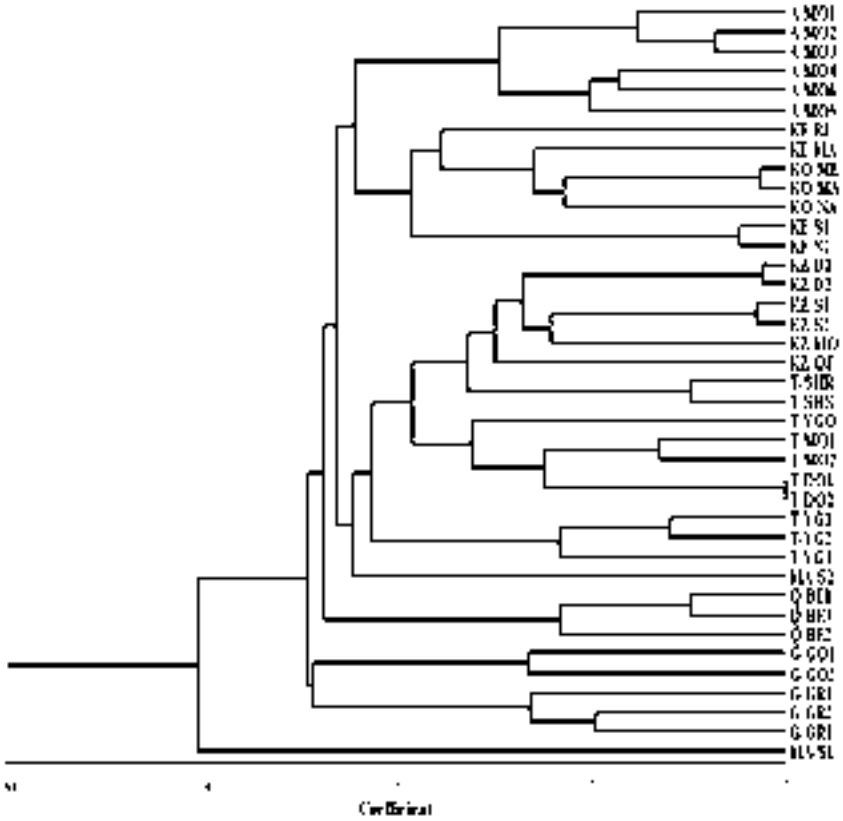

Figure 3. Dendrogram of individual populations of *Common reed*structured on the basis of RAPD data, by UMGMA method, resulting from similarity matrix calculated with the metric of Nie & Li.

Another interesting attempt was the comparison of genetic similarities within the provinces. Geographic distance between KZ-D1 and KZ-D2 was less than 10 km and genetic similarity between these populations was 0.84. All populations from Qom (Q-

BE1, Q-BE2 and Q-BE3) were placed in the same cluster. Genetic distances among these populations were less because Qom is a relatively small province with a small variability in climatic conditions. Also, genetic distances among populations from Ardrbil (A- MO1, A-MO2, A-MO3, A-MO4, A-MO5 and A-MO6) were small. Genetic distances among populations from Tehran were large and this is due to climatic variation of Tehran. T-SHR and T-SHS populations were from south , T-DO1, T-DO2, T-MO1 and T-MO2 populations were from northwest and T-VGO, T-VG1, T-VG2 and T-VG3 populations were from southeastern Tehran. Koppitz (1999) detected lower genetic diversity among samples from any location, with coefficients ranging form 0.10 to 1.0 in general, also the genetic distance of investigated common reed samples within Europe increase by increase in geographic distance. Study of genetic variation of *Common reed*of the Charles River, Watershed, Massachustts, showed that populations grouped geographically with distance along the river, indicative of dispersal in water (Keller, 2000).

The first and second PCA axes of RAPD data accounted for 8.29 % and 6.40 % of the total variation, respectively, concluding that PCA based on RAPD data did not efficiently distinguish between populations (Figure 4).

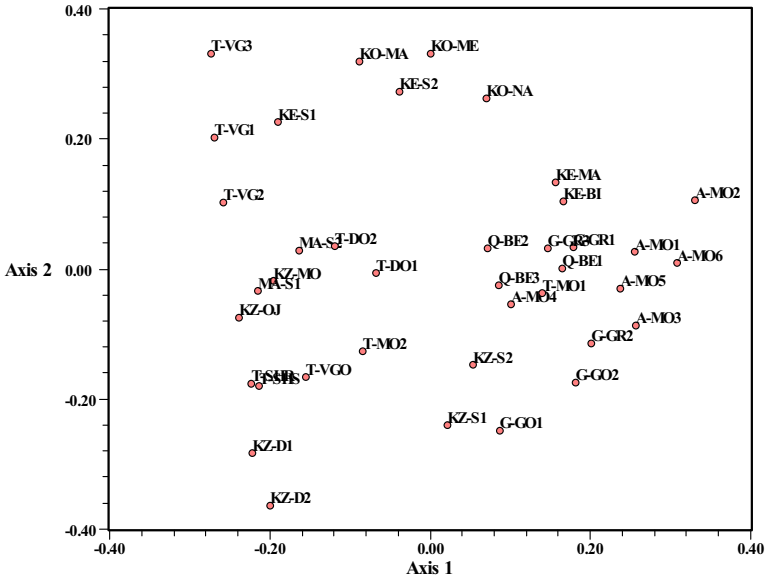

Figure 4. PCA scores of populations of *Common reed*based on RAPD data.

The PCR-fingerprinting data strongly support a primarily vegetative mode of reproduction and spread. These results support the clonal nature of Common reed along with its growth (growth pattern) and primarily vegetative propagation. Gene flow by means of dispersal of vegetative propagules plays an important role in providing variation among or within clonal population (McLellan *et al*., 1997). Both isozyme and RAPD analysis revealed genetic diversity in Giant reed (*Arundo donax*), suggesting that asexual reproduction is the primary means of spread of this weed (Khudamrongsawat *et al*., 2004) in the Santa Ana River, California. Our results have also supported the clonal nature of Common reed with its growth and spread primarily by vegetative propagation, because spread of studied populations occur mainly asexually. Management efforts should focus on preventing establishment and spread of vegetative propagules. A high level of clonal diversity was indicated in *Common reed*with PD (Proportion distinguishable variations, PD = 0.53) in the Yellow River delta in China (Guo *et al*., 2003). Cluster analysis of genetic similarity coefficients range form 0.30 to 0.80 demonstrating a high degree of genetic diversity among common reed stands world wide (Koppitz, 1999).

Much information on the genetic structure and diversity of common reed populations has been gained using the RAPD technique. RAPD is an effective method to detect intra and inter population variation and is still widely being used for such purposes in many plants (Koppitz *et al*., 1997; Koppitz, 1999; Keller, 2000; Kuhl *et al*., 1999; Bussell *et al*., 2005; Curn *et al*., 2007).

Finally, our results indicate a high diversity in the populations of *Common reed*in Iran. This high level of genetic diversity shows the need to find a way to facile management of genetic resources. Moreover, information concerning the geographical and taxonomic distribution of genetic variation provides guidelines for different sampling strategies of populations. It is possible that these variations among populations will affect successful management of *Common reed* using chemical or the other methods of control.

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