Original article

Evaluation of genetic diversity of *Panicum turgidum* Forssk from Saudi Arabia

Abdulaziz M. Assaeed [a], Sulieman A. Al-Faifi [a], Hussein M. Migdadi [a,*], Magdy I. El-Bana [b], Abdulaziz A. Al Qarawi [a], Mohammad Altaf Khan [a]

[a] *Department of Plant Production, King Saud University, PO Box 2460, Riyadh 11451, Saudi Arabia*
[b] *Department of Botany, Faculty of Science, Port Said University, Egypt*

ARTICLE INFO

ABSTRACT

The genetic diversity of 177 accessions of *Panicum turgidum* Forssk, representing ten populations collected from four geographical regions in Saudi Arabia, was analyzed using amplified fragment length polymorphism (AFLP) markers. A set of four primer-pairs with two/three selective nucleotides scored 836 AFLP amplified fragments (putative loci/genome landmarks), all of which were polymorphic. Populations collected from the southern region of the country showed the highest genetic diversity parameters, whereas those collected from the central regions showed the lowest values. Analysis of molecular variance (AMOVA) revealed that 78% of the genetic variability was attributable to differences within populations. Pairwise values for population differentiation and genetic structure were statistically significant for all variances. The UPGMA dendrogram, validated by principal coordinate analysis-grouped accessions, corresponded to the geographical origin of the accessions. Mantel's test showed that there was a significant correlation between the genetic and geographical distances ($r = 0.35$, $P < 0.04$). In summary, the AFLP assay demonstrated the existence of substantial genetic variation in *P. turgidum*. The relationship between the genetic diversity and geographical source of *P. turgidum* populations of Saudi Arabia, as revealed through this comprehensive study, will enable effective resource management and restoration of new areas without compromising adaptation and genetic diversity.

© 2017 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Saudi Arabia has approximately 170 million hectares of rangelands, which represent approximately 70% of its territory. However, a major portion of this area is degraded due to overgrazing and other human activities (Al-Rowaily, 1999). This overexploitation of rangeland resources has resulted in biodiversity loss and habitat fragmentation (Khan et al., 2012).

Conserving important native rangeland species is necessary to increase the availability of forage plants adapted to the various edaphic and climatic conditions of different regions of the country and to develop plant material for the restoration of deteriorated rangelands. With global climate change, continuous pressure from abiotic factors (e.g., extreme temperature and drought), and anthropological activities impacting on native vegetation, conservation of grass species such as *Panicum turgidum* may be a potential candidate for forage production and habitat restoration.

Panicum is the largest genus within *Poaceae*, a diverse family consisting of approximately 450 annual and perennial species (Aliscioni et al., 2003). *Panicum* is distributed in North Africa, extending eastward from Mauritania and Senegal through the Sahara and Sahel to Sudan and the horn of Africa and western Asia to Pakistan and India (Ashraf et al., 2006; Batanouny, 2001; Le Houérou, 1987; Osman et al., 2008; Sinha et al., 2009). In Saudi Arabia, it grows in various habitats ranging from the seashore, inte-

---

rior desert plains, and sand dunes to the mountain ranges of the southwest (Chaudhary and Al-Juwaid, 1999). It was noted that high-yielding grazing species (Khan et al., 2009) can endure harsh climate (Oatham, 1997) and may have potential as a nurse plant to other important species in restoration projects (Anthelme et al., 2007; Anthelme and Michalet, 2009; El-Keblawy et al., 2011).

P. turgidum is a C4 perennial halophytic grass, widely distributed in saline and arid areas, highly palatable and relatively high in crude protein content (Heneidy, 2000). Despite the importance of *P. turgidum* for grazing and desert restoration, the genetic variation of this species in Saudi Arabia has not been characterized.

Genetic variation is the basic element of biological polymorphism and species diversity. It provides a guide for the selection of appropriate germplasm for genetic improvement and or restorations activities, as well as predicting the degree of inheritance and the levels of heterosis in various hybrid combinations. Molecular markers have become the method of choice for the assessment of genetic diversity in plants. Studies applying molecular markers to access the genetic variability and phylogenetic relationships in *P. turgidum* populations are limited, and to our knowledge, there are no publications reporting the investigation of genetic diversity of *P. turgidum* using molecular markers. Amplified fragment length polymorphism (AFLP) (Vos et al., 1995) is a reliable, robust, and highly reproducible assay for dominant genetic markers and has been widely used for genetic studies in many organisms, including cultivated plants and their wild relatives. In this study, we aimed to evaluate the genetic diversity of *P. turgidum* collected from different habitats in Saudi Arabia.

2. Materials and methods

2.1. Sample collection and DNA extraction

A total of 177 *P. turgidum* accessions representing collections from 10 wild populations (11–28 accessions per population) from different habitats in Saudi Arabia were randomly selected. The longitude, latitude, and elevation of these populations are listed in Table S1 (available online). One fresh leaf from each accession was collected, dropped in liquid N_2, and stored at $-80\,°C$ until DNA isolation. DNA was extracted using the modified sodium dodecyl sulfate protocol (Hoelzel, 1998). The quality and concentration of the extracted DNA was assessed using 0.8% agarose gel electrophoresis and spectrophotometry. Samples were diluted with Tris-EDTA (TE) buffer, and the concentration was fixed at a 100 ng/μL.

2.2. AFLP procedure

The AFLP plant-mapping guide from (ABI) Applied Bio-systems (Waltham, MA, USA) was adapted in this study, with some modifications. Samples (5 μL; 0.5 μg of genomic DNA) were added to 0.2-mL tubes, and 5.5 μL of the digestion-ligation mix was added to each sample, mixed well, and incubated at $37\,°C$ for 3 h. $TE_{0.1}$ (90 μL) was added to each tube and stored at $4\,°C$. The first polymerase chain reaction (PCR) was performed with primers that matched the adapter sequence and had one additional "selective" base (EcoR1 primer [EcoR1 + A] and MseI primer [MseI + C]). In a 1.5 ml tube, the PCR mix was formulated with 10 μL of 2 × PCR Master Mix (Promega, Madison, USA), 0.2 μM of each primer MseI-C and EcoRI-A, 3 μL of diluted dig-lig, and double-distilled water, to a final volume of 20 μL. The PCR parameters were as follows: 30 cycles of $72\,°C$ for 2 min, $94\,°C$ for 30 s, $56\,°C$ for 30 s, $72\,°C$ for 2 min, followed by $60\,°C$ for 10 min and a hold at $10\,°C$. The product was then diluted 10 times with TE. For the final PCR reaction, the amplified +1 product was used as the new template.

The primers for this reaction had the same sequence as the +1 primer except for two additional selective bases. The EcoR1 + 2 primers were labeled with a fluorescent dye (6-FAM) that could be detected with the ABI 3130xl Genetic Analyzer. Initially, 12 EcoR1 + 2/MseI + 3 primer combinations were tested individually to identify the best amplifications (most readable and numerous polymorphic bands). The PCR parameters were as follows: initial denaturation at $94\,°C$ for 2 min, followed by 12 cycles of $94\,°C$ for 30 s, $65\,°C$ for 30 s with a gradient reduction of $0.7\,°C$ per cycle, and $72\,°C$ for 1 min. This was followed by 23 cycles of $94\,°C$ for 30 s, $56\,°C$ for 30 s, $72\,°C$ for 1 min, and a final extension of $72\,°C$ for 10 min.

2.3. Running the AFLP gel on the ABI 3130xl genetic analyzer

One-microliter of the PCR-amplified product was mixed with 0.5 μL of GeneScan 500 LIZ size standard (Applied Biosystems P/N 4322682) and 8.5 μL of Hi-Di Formamide (Applied Biosystems P/N 4311320). The mixture was denatured and loaded on the 16-capillary system of the Applied Biosystems 3130xl Genetic Analyzer. A 36-cm capillary array (Applied Biosystems P/N 4315931) and 3130 POP-7 polymer (Applied Biosystems P/N 4352759) were used.

2.4. Data analysis

AFLP fragments were analyzed with the GeneMapper Analysis Software v3.7 (ABI), and the data were assembled in binary format (allele presence [1] or absence [0]). The threshold for allele calling was set at 100 relative fluorescence units (rfu), so that any peaks at 100(rfu) or higher were assigned a "1" and those that were lower were assigned a "0." Fragment analysis was carried out for allele sizes in the range of 100–500 bp. Loci with band frequencies higher than $1 - (3/N)$, where N = number of individuals sampled, were deleted to account for estimation bias due to overestimation of parameters by as much as 5%, especially in the case of small sample sizes (Alexander et al., 2004; Lynch and Milligan, 1994). The data generated from the AFLP analysis were analyzed using Jaccard similarity coefficient (Jaccard, 1908). The resulting similarity coefficients were used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) employing the PAST3 program. The polymorphism information content (PIC) for each primer was calculated to estimate its allelic variation as follows: $PIC = 1 - \sum_{j=1}^{n} Pij^2$, where Pij is the frequency of the ith allele for marker j and the summation extends over n alleles, calculated for each AFLP marker (Anderson et al., 1993). The power of discrimination was calculated using the formula $PD = 1 - \sum gi^2$, where gi is the frequency of the ith genotype (Kloosterman et al., 1993). The binary matrix of presence/absence was used for further analysis with GenAlex 6.5 complement for MSExcel (Peakall and Smouse, 2012). The total number of alleles, genetic diversity, and Shannon index for each population, and the number of private alleles per population and region were calculated using this approach. Genetic differentiation between populations was determined using phiPT, a measure that allows intra-individual variation to be suppressed and is therefore ideal for comparing codominant and binary data, with 10,000 permutations. Analysis of molecular variance (AMOVA) among and within populations and geographical regions was performed using GenAlex (Peakall and Smouse, 2012). Group analysis of populations based on Gower genetic distance was also conducted. Correlations between populations and geographic distances were assessed using Mantel's test with 5000 permutations (Mantel, 1967), performed using GenAlex (Peakall and Smouse, 2012).

3. Results

The four AFLP primer combinations revealed 836 polymorphic amplified DNA fragments. The number of amplified DNA fragments ranged from 143 for primers EcoR1-AA/Mse1-CCA to 262 for EcoR1-CT/Mse1-CAG. The average number of polymorphic fragments per accession was 37.5 and ranged between 31.8 and 51.8 for primer pair combinations EcoR1-AA/Mse1-CAG and EcoR1-CT/Mse1-CAG, respectively). An average of 209 polymorphic fragments per primer combination was obtained across the accessions. All the primers showed 100% polymorphism, and the overall size ranged from 100 to 500 bp.

The four primers generated 26,937 bands, with an average of 6734 bands per primer combination across accessions (range: 5622 for primer pair combination EcoR1-AA/Mse1-CAG to 9166 for primer pair combination EcoR1-CT/Mse1-CAG). All primer combinations produced high polymorphism information content (PIC) and power of discrimination (PD) values (Table 1).

The genetic diversity parameters analyzed in this study are presented in Table 2. The average number of alleles found within each population (N_a) was 1.32 (range: 1.01 in the Madinah population to 1.65 in Farasan), and the average number of effective alleles was 1.33 (range: 1.20–1.45). Nabhaniya showed lower values for both the Shannon index and heterozygosity compared with the other populations. Uqair and Farasan showed the highest values of Shannon index and heterozygosity. All populations showed a mean value of 0.31 for the Shannon index and 0.20 for heterozygosity. The Farasan population showed the highest polymorphic percentage (82.42%). The average polymorphic value observed was 66.15% (range: 50.72%–82.42%). Six private alleles were obtained, of which four were presented in the accessions collected from the Farasan population and one each from the Uqair and Taif populations.

With respect to the geographic regions, populations collected in the southern region (Dhabia and Farasan) showed the highest genetic diversity (average number of different alleles, 1.87; number of effective alleles, 1.44; Shannon index, 0.42; heterozygosity value, 0.27 with ten private alleles; and polymorphic percentage, 93.30%), while the central region showed the lowest values for the number of different and effective alleles, Shannon index, heterozygosity, and polymorphism percentage (Table 2).

The Analysis of Molecular Variance (AMOVA) indicated that most of the genetic variation was attributable to differences within populations (78%), while the variability among populations and among regions contributed 10% and 12%, respectively (Table 3). Population differentiation and genetic structure were highly significant for all variances. The pairwise Jaccard genetic similarity among the 177 accessions ranged from 0.0 to 0.78 (Table S3, available online). The population genetic distance values ranged from 0.18 (between Nabhaniya and Thumama) to 0.55 (between Rwag and Farasan populations; Table S2, available online).

Cluster analysis using Jaccard coefficient supported by PCoA led to near agglomerations (Figs. S1 and S2, available online). Mantel test resulted in a cophenetic correlation coefficient of 0.934 for the Jaccard coefficient. The UPGMA dendrogram revealed clusters that almost corresponded to the geographical origin of the accessions. The 177 accessions were distributed among eight clusters (Table 4). Of the eight clusters, five were considered main clusters and compassed 163 accessions, representing 92% of the total accessions. Sixty-six accessions (37%) were grouped to form the first main cluster. These accessions were collected from the central part of the country and had a contribution of 77% (51 accessions); 9 accessions (14%) were from the eastern part, and 6 accessions (9%) from the southern part. In the second main cluster, 54 accessions (31%) were collected from the eastern part (18) with 34%, 29 accessions (54%) from the southern part, and 7 accessions (13%) from the western part of the country. The other three clusters showed lower distributions (10%, 9%, and 6%). Accessions collected from the western part were distributed in two clusters; 17 of 28 accessions formed one cluster and 4 accessions were grouped in the second cluster. The remaining 3 clusters compassed accessions collected from the central part of Saudi Arabia, with 13 out of 14 accessions (93%).

Group analysis of populations is presented in Fig. 1. Pairwise Population Matrix of Genetic distance is presented in supplementary Table S2 available online. Populations were grouped according to their geographic region. Three clusters with high bootstrap values were formed. Accessions from the western parts of the country (Taif and Medina) formed the first cluster. The second cluster comprised all populations collected from the central region of the country and one population from the eastern region, and finally, two populations from the western region (Farasan and Dhabia) were grouped with one eastern population in the third cluster. Mantel test revealed that there was a significant correlation between the pairwise geographical and genetic distances ($r = 0.35$, $P < 0.04$) for all the populations. Principal coordinate analysis (PCoA) further validated the results of the cluster analysis (Fig. 2). In PCoA, the first two components explained more than 63.6% of the variation in the estimates of genetic similarity. Altogether, three distinct groups were revealed by the first two principal coordinates. Group 1 included all accessions from Madinah and Taif (western parts of country). The accessions from Uqair, Farasan, and Dhabia were clearly differentiated and formed group 2. Group 3 included accessions from Nabhaniya, Thumama, Ras Tanura, Sajir, and Rwag.

4. Discussion

In the present study, four AFLP EcoRI + MseI primer combinations generated 100% polymorphism, which was confirmed by analysis of the individual DNA profiles and genetic diversity. These primers generated 836 differently sized AFLP markers (alleles)

Table 1
Characteristics of AFLP primers selected in *Panicum turgidum* genetic diversity.

Primer combination EcoR1/MseI	Total alleles[a]	Total no. of bands[b]	Average bands[c] (range)	PIC	DP
AA/CAC	258	6282	35.5 (4-114)	0.99	0.99
AA/CAG	173	5622	31.8 (4-104)	0.99	0.99
AA/CCA	143	5867	33.1 (4-126)	0.99	0.99
CT/CAG	262	9166	51.8 (4-117)	0.99	0.99
Total	836	26937	–	–	–
Average	209	6734	37.5	0.99	0.99

PIC; polymorphic information content, DP; discrimination power.
[a] Total number of differently sized AFLP markers amplified across all accessions.
[b] Total number of AFLP markers (data points) scored for all accessions.
[c] Average number of AFLP markers scored per accession.

Table 2
Diversity parameters of *Panicum turgidum* populations obtained from the analysis of 836 AFLP alleles.

Population	# of accessions	Na	Ne	I	H	%P	Private alleles
Nabhaniya	28	1.17	1.20	0.22	0.13	58.37	0
Sajir	15	1.19	1.33	0.30	0.20	59.57	0
Thumama	22	1.34	1.30	0.29	0.19	66.99	0
Rwag	14	1.21	1.29	0.28	0.18	60.29	0
Ras Tanura	11	1.10	1.29	0.27	0.18	54.90	0
Dhabia	21	1.53	1.37	0.35	0.23	76.56	0
Uqair	15	1.51	1.45	0.39	0.26	75.72	1
Farasan	23	1.65	1.40	0.38	0.24	82.42	4
Taif	15	1.52	1.38	0.36	0.23	75.96	1
Madinah	13	1.01	1.27	0.25	0.16	50.72	0
Mean		1.32	1.33	0.31	0.20	66.15	
Min	11	1.01	1.20	0.22	0.13	50.72	
Max	28	1.65	1.45	0.39	0.26	82.42	
Region							
Central	79	1.59	1.30	0.30	0.19	79.31	2
East	32	1.80	1.40	0.39	0.25	90.19	1
South	38	1.87	1.44	0.42	0.27	93.30	10
west	28	1.63	1.35	0.34	0.22	81.70	11
Mean		1.72	1.37	0.36	0.23	86.12	
Min	28	1.59	1.30	0.30	0.19	79.31	
Max	79	1.87	1.44	0.42	0.27	93.30	

Na = # of different alleles, Ne = # of effective alleles, I = shannon index, H = diversity = $1 - (p^2 + q^2)$, # private alleles = # of alleles unique to a single population (region).

Table 3
Summary of AMOVA. Estimated variance (Est.Var) percentage and the differentiation among region, among populations and within population values for *Panicum turgidum* using AFLP markers.

Source	df	MS	Est. Var.	%
Among regions (AR)	3	825.44	13.16	12
Among pops (AP)	6	278.50	10.99	10
Within pops (WP)	167	87.75	87.75	78
Total	176		111.90	100
PhiRT (value/*P*<)	0.118/0.001			
PhiPR	0.111/0.001			
PhiPT	0.216/0.001			

PhiRT = AR/(WP + AP + AR) = AR/TOT, PhiPR = AP/(WP + AP), PhiPT = (AP + AR)/(WP + AP + AR) = (AP + AR)/TOT. AR = Est. Var. Among Regions, AP = Est. Var. Among Pops, WP = Est. Var. Within Pops.

Table 4
Clustering patterns of collected *Panicum turgidum* accessions based on AFLP data.

Location	Region	No. of accessions	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
Nabhaniya	Central	28	14			6		3	4	1
Thumama	Central	22	12			7		2		1
Rwag	Central	14	14							
Sajir	Central	15	11			2				2
Ras Tanura	East	11	9			1		1		
Uqair	East	21		18			3			
Dhabia	South	15	6	8			1			
Farasan	South	23		21			2			
Taif	West	15		7			1			
Madinah	West	13			10		3			
Total (frequency)		177	1.00	66 0.37	54 0.31	17 0.10	16 0.09	10 0.06	6 0.03	4 0.02	4 0.02
Central		79	0.45	51 0.77			15 0.94		5 0.84	4 1.00	4 1.00
East		32	0.18	9 0.14	18 0.34		1 0.06	3 0.30	1 0.16		
South		38	0.21	6 0.09	29 0.54			3 0.30			
West		28	0.16		7 0.13	17 1.00		4 0.40			

across all accessions, which was considered sufficient to estimate the diversity of *P. turgidum*. The number of alleles obtained in this study was more than that obtained in previous studies on the genetic diversity of *P. virgatum* (452 polymorphic AFLP markers) by Todd et al. (2011), 91 RAPD markers by Gunter et al. (1996), 63 EST and genomic SSR loci by Narasimhamoorthy et al. (2008), and 16 EST-SSR loci by Cortese et al. (2010). Mohammadi and Prasanna (2003) indicated that the percentage of polymorphic markers becomes reliable only when a large number of loci are generated. However, they did not indicate the specific marker numbers required for this kind of study.

In this study, the average number of polymorphic fragments was 37.5 per primer pair combination, which exceeded that of previous assessments of genetic diversity in *Panicum* accessions. The
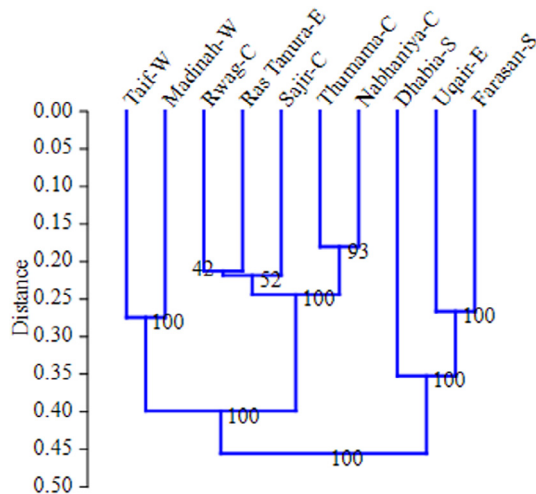
Fig. 1. Dendrograms generated using unweighted pair group method with arithmetic average (UPGMA) analysis, showing relationships between 10 *Panicum turgidum*, populations using AFLP data based on Gower genetic distance.

large number of polymorphic DNA fragments revealed by AFLP is an advantage of the technique, as the accuracy of measurements of genetic distances increases with the number of loci used (Travis et al., 1996). In previous studies, 18.2 loci based on 5 RAPD primers (Gunter et al., 1996) and 17.8 loci from 7 RAPD primers (Casler et al., 2007) were reported. The high polymorphism percentage (100%) recorded in this study was consistent with several previous reports using different types of markers in *Panicum* sp. Nageswara-Rao et al. (2013) reported 84.6%–100% polymorphism using RAPD markers, and Huang et al. (2011) reported 88.2%–100% polymorphism in sequence-related amplified polymorphic (SRAP) markers and 85.71%–100% polymorphism in expressed sequence tags-simple sequence repeats (EST-SSRs). In another study, Ranamukhaarachchi et al. (2000) used a modified AFLP technique in sea oats and amplified 95 bands with 8 AFLP primers, of which 52 were polymorphic. Subudhi et al. (2005) used 12 AFLP *Eco*RI + *Mse*I primer combinations and generated 42%–81% polymorphisms with a mean of 59% in sea oats. Molecular analysis of

87 samples of *P. coloratum* with 7 ISSR and 10 SSR primers generated 88% and 100% polymorphic loci, respectively (Armando et al., 2015). In comparison, the present study detected a higher rate of polymorphism and provided higher resolution owing to the generation of a large number of loci utilizing electrophoresis system using FAM labeled and laser detectors in ABI genetic analyzer have much higher detection power compared with ordinary PAGE systems used in other studies.

The PIC values, which are a reflection of allelic diversity and frequency among the genotypes, are estimators of the usefulness of any marker system for genotype distinction and genetic diversity analysis. Based on the polymorphism value, Vaiman et al. (1994) and Xie et al. (2010) classified PIC values as high (>0.5), medium (between 0.25 and 0.5), or low (<0.5). In this study, the four primer combinations generated a high PIC value (0.99), indicating that the AFLP markers could be used to develop a high loci polymorphism database for *P. turgidum*. The mean PIC and PD values of the primer combinations in this study were higher than those reported in the earlier study involving *Panicum* sp. (Narasimhamoorthy et al., 2008).

To our knowledge, this study is the first comprehensive survey of genetic diversity in *P. turgidum* accessions of Saudi Arabia using AFLP markers. The wide range of similarity among the accessions and among populations exceeded that of many studies using other *Panicum* spp. Cortese et al. (2010) reported a range from 0.76 to 0.97 using EST-SSR. Missaoui et al. (2006) recorded a range of 0.70–0.82 using RFLP markers, and Gunter et al. (1996) reported a range of 0.53–0.78 using RAPD markers. The greater degree of genetic diversity among the accessions and populations in our study could be attributed to their being more accessions from different habitats and altitudes and a more alleles detected using the AFLP marker system in our study. A previous study comparing the efficiency of different marker systems in detecting genetic diversity revealed that EST-SSRs and RFLPs are both highly conserved and therefore, might result in the generation of smaller genetic distances than a dominant marker system. These authors concluded that AFLP was the best-suited molecular assay for fingerprinting and for assessing genetic relationships with high accuracy (Garcia et al., 2004).
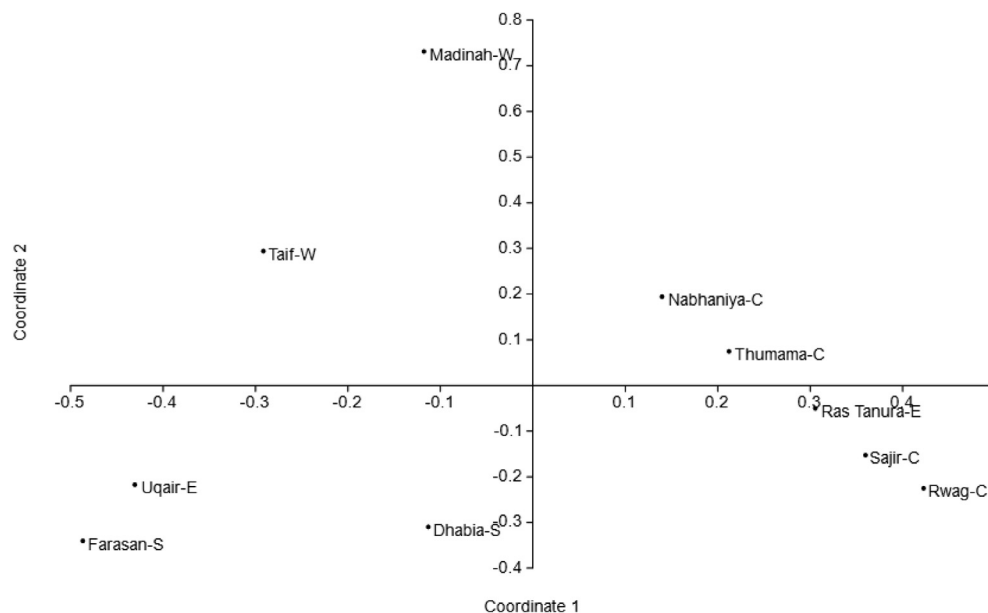
Fig. 2. Principal coordinate analysis (PCoA) of the 10 *Panicum turgidum* populations based on AFLP markers. E: east, W: west, C: central of the Saudi Arabia.

Cluster analysis using the UPGMA approach, verified by PCoA, showed that the differentiation of genetic structure followed a geographic pattern. Strong bootstrap support was obtained for the branches, indicating the reliability and stability of the inferred relationships as well as the robustness of the AFLP dataset. The goodness-of-fit of the AFLP-generated dataset for the cluster analysis was also supported by the high cophenetic correlation coefficient (0.934) separating the population according to its origin. The results were also supported by the significant positive correlation between the populations and the geographical pairwise distances ($r = 0.45$, $P < 0.04$) using Mantel's test.

Although there were some exceptions, within each cluster, accessions from the same geographical origins tended to group together, and the overall geographic proximity was high, as indicated by the AFLP dendrogram. These findings are consistent with the results of a previous study indicating limited gene flow among populations due to linear and fragmented distribution (Berg and Hamrick, 1997). These differences could be caused by adaptation to geographic regions. Other studies have revealed that most of the germplasm examined subcluster according to the adaptive regions (Casler et al., 2007; Narasimhamoorthy et al., 2008; Zalapa et al., 2010). Loveless and Hamrick (1984) have listed a number of evolutionary factors that affect the population diversity and structure of a species, including the reproductive system, pollen or seed dispersal mechanism, geographic range, successional status, and natural selection. Young et al. (2000) have reported that seed dispersal over a large area leads to greater gene flow and has a more homogenizing effect on the genetic variation among populations, while mechanisms that lead to seed dispersal over very short distances cause an increase in the genetic structuring of populations. Overall, accessions collected from the Uqair and Farasan populations showed high genetic variability, while accessions collected from Nabhaniya and Madinah showed lower genetic diversity. In addition, Farasan recorded four private alleles, while both Uqair and Taif recorded one private allele for each location. The presence of accession-specific bands suggested distinctness and limited gene flow (Armando et al., 2015). Accordingly, both the eastern and southern regions harbored more genetic variability, and the populations of these regions recorded high values of genetic diversity parameters. The accessions (84%) collected from the southern region were distributed to two main clusters, while 64% of the accessions collected from the eastern regions could be grouped into two main clusters.

The amount of genetic variation among and within populations has been demonstrated to be closely associated with the breeding system, seed dispersal mechanism, and geographic distribution range (Nybom and Bartish, 2000). The mean within-population diversity has been shown to be an accurate predictor of the total within-species diversity (Hamrick and Godt, 1989). In our study, most of the genetic variation was attributable to differences within populations (78%) associated with high and significant pairwise values for population differentiation and genetic structure (0.216, $P < 0.001$). These results are consistent with those of Narasimhamoorthy et al. (2008) who reported that 79.6% of the total variance in *P. virgatum* was attributable to within-population diversity. Greater within-population variation has been reported in different perennial grasses, including *Phalaris aquatica* (74%; Mian et al., 2005) and *Chloris gayana* Kunth (88%, Ubi et al., 2003), using AFLP markers. In RAPD-based studies for evaluating population differentiation in outcrossing taxa, have demonstrated that among-population diversity was closely correlated with maximum geographic distance between sampled populations and long-lived taxa (Nybom and Bartish, 2000). In outcrossing, late successional taxa retain most of their genetic variability within populations. By contrast, annual, selfing, and/or early successional taxa allocate most of the genetic variability among populations. On the

other hand, using ISSR markers, 77% of the molecular variability in *P. coloratum* was found to be attributable to accession differences; however, SSR data detected only 37% molecular variability that could be attributable to differences between accessions (Armando et al., 2015).

5. Conclusions

In this study, AFLP analysis revealed a large number of distinct, scorable fragments per primer pair. The AFLP technique, along with clustering algorithms, PCoA, Mantel test, and AMOVA analysis, showed a high-level of genetic diversity within Saudi *P. turgidum* populations. This diversity should be maintained in order to prevent potential extinction or loss of genetic diversity of this important species. AMOVA suggested high diversity within populations, which allows for breeding and selection within each population.

Conflict of interest

The authors have declared that no conflict of interest exists. All roles and conditions of publication are accepted by authors and their responsibilities to follow.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sjbs.2017.04.002.

References

Alexander, D.E., Kaczorowski, D.J., Jackson-Fisher, A.J., Lowery, D.M., Zanton, S.J., Pugh, B.F., 2004. Inhibition of TATA binding protein dimerization by RNA polymerase III transcription initiation factor Brf1. J. Biol. Chem. 279, 32401–32406.

Aliscioni, S.S., Giussani, L.M., Zuloaga, F.O., Kellogg, E.A., 2003. A molecular phylogeny of Panicum Poaceae: Paniceae: tests of monophyly and phylogenetic placement within the Panicoideae. Am. J. Bot. 90, 796–821.

Al-Rowaily, S.L., 1999. Rangeland of Saudi Arabia and the "tragedy of commons". Rangelands 3, 27–29.

Anderson, J., Churchill, G., Autrique, J., Tanksley, S., Sorrells, M., 1993. Optimizing parental selection for genetic linkage maps. Genome 36 (1), 181–186.

Anthelme, F., Michalet, R., 2009. Grass-to-tree facilitation in an arid grazed environment Aïr Mountains, Sahara. Basic Appl. Ecol. 105, 437–446.

Anthelmea, F., Michaletb R., Saadou, M., 2007. Positive associations involving the tussock grass *Panicum turgidum* Forssk in the Air-Tenere Reserve, Niger. J. Arid Environ. 68, 348–362.

Armando, L.V., Tomás, M.A., Garayalde, A.F., Carrera, A.D., 2015. Assessing the genetic diversity of Panicum coloratum var. makarikariense using agromorphological traits and microsatellite-based markers. Ann. Appl. Biol. 167, 373–386.

Ashraf, M., Hameed, M., Arshad, M., Ashraf, Y., Akhtar, K., 2006. Salt tolerance of some potential forage grasses from Cholistan Desert of Pakistan. In: Khan, M.A., Weber, D.J. (Eds.), Ecophysiology of High Salinity Tolerant Plants Tasks for Vegetation Science, vol. 40. Springer Verlag, Dordrecht, pp. 31–54.

Batanouny, K.H., 2001. Plants in the deserts of the Middle East. In: Cloudsley-Thompson, J.L. (Ed.), Adaptation of Organisms to the Desert. Springer Verlag, Heidelberg, p. 193.

Berg, E.E., Hamrick, J.L., 1997. Quantification of genetic diversity at allozyme loci. Can. J. Forest Res. 27, 415–424.

Casler, M.D., Stendal, C.A., Kapich, L., Vogel, K.P., 2007. Genetic diversity, plant adaptation regions, and gene pools for switchgrass. Crop Sci. 47, 2261–2273.

Chaudhary, S.A., Al-Juwaid, A.A., 1999. Vegetation of the Kingdom of Saudi Arabia. National Agriculture Research Center, Ministry of Agriculture & Water Riyadh, Saudi Arabia.

Cortese, L.M., Honig, J., Miller, C., Bonos, S.A., 2010. Genetic diversity of twelve switchgrass populations using molecular and morphological markers. Bioenergy Res. 3, 262–271.

El-Keblawy, A., Al-Ansari, F., Al-Shamsi, N., 2011. Effects of temperature and light on salinity tolerance during germination of the two desert glycophytic grasses *Lasiurus scindicus* and *Panicum turgidum*. Grass Forage Sci. 66, 173–182.

Garcia, A.A.F., Benchimol, L.L., Barbosa, A.M.M., Geraldi, I.O., Souza-Jr, C.L., de Souza, A.P., 2004. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. Gen. Mol. Biol. 274, 579–588.

Gunter, L.E., Tuskan, G.A., Wullschleger, S.D., 1996. Diversity among populations of switchgrass based on RAPD markers. Crop Sci. 364, 1017–1022.

Hamrick, J.L., Godt, M.J.W., 1989. Allozyme diversity in plant species. In: Brown, A.H. D., Clegg, M.T., Kahler, A.L., Weir, B.S. (Eds.), Plant Population Genetics, Breeding and Genetic Resources. Sinauer, Sunderland, MA, pp. 43–63.

Heneidy, S.Z., 2000. Palatability, chemical composition and nutritive value of some common range plants from Bisha, Region, Southwestern of Saudi Arabia Desert. Inst. Bull. Egypt 2, 345–360.

Hoelzel, A.R., 1998. Genetic structure of cetacean populations in sympatry, parapatry and mixed assemblages; implications for conservation policy. J. Hered. 89, 451–458.

Huang, L.K., Bughrara, S.S., Zhang, X.Q., Bales-Arcelo, C.J., Bin, X., 2011. Genetic diversity of switchgrass and its relative species in Panicum genus using molecular markers. Biochem. Syst. Ecol. 39, 685–693.

Jaccard, P., 1908. Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44, 223–270.

Khan, M.A., Ansari, R., Ali, H., Gul, B., Nielsen, B.L., 2009. *Panicum turgidum*, potentially sustainable cattle feed alternative to maize for saline areas. Agric. Ecosyst. Environ. 129, 542–546.

Khan, S., Al-Qurainy, F., Nadeem, M., 2012. Biotechnological approaches for conservation and improvement of rare and endangered plants of Saudi Arabia. Saudi J. Biol. Sci. 191, 1–11.

Kloosterman, A.D., Budowle, B., Daselaar, P., 1993. PCR amplification and detection of the human D1S80 VNTR locus amplification conditions, population genetics and application forensic analysis. Int. J. Legal Med. 105, 257–264.

Le Houérou, H.N., 1987. Indigenous shrubs and trees in the silvopastoral systems of Africa. In: Steppler, H.A., Nair, P.K.R. (Eds.), Agroforestry: A Decade of Development International Centre for Research in Agroforestry. Nairobi, Kenya, pp. 139–151.

Loveless, M.D., Hamrick, J.L., 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Evol. Syst. 15, 65–96.

Lynch, M., Milligan, B., 1994. Analysis of population-genetic structure using RAPD markers. Mol. Ecol. 3, 91–99.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209–220.

Mian, M.A.R., Zwonitzer, J.C., Chen, Y., Saha, M.C., Hopkins, A.A., 2005. AFLP diversity within and among hardinggrass populations. Crop Sci. 45, 2591–2597.

Missaoui, A.M., Paterson, A.H., Bouton, J.H., 2006. Molecular markers for the classification of switchgrass *Panicum virgatum* L germplasm and to assess genetic diversity in three synthetic switchgrass populations. Genet. Resour. Crop Evol. 53, 1291–1302.

Mohammadi, S.A., Prasanna, B.M., 2003. Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Sci. 43, 1235–1248.

Nageswara-Rao, M., Stewart, C.N., Kwit, C., 2013. Genetic diversity and structure of natural and agronomic switchgrass *Panicum virgatum* L populations. Genet. Resour. Crop Evol. 60, 1057–1068.

Narasimhamoorthy, B., Saha, M., Swaller, T., Bouton, J., 2008. Genetic diversity in switchgrass collections assessed by EST-SSR markers. Bioenergy Res. 1, 136–146.

Nybom, H., Bartish, I., 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect. Plant Ecol. Evol. Syst. 3, 93–114.

Oatham, M.P., 1997. Water relations of *Zygophyllum hamiense*, *Heliotropium kotschyi* and *Panicum turgidum* and their response to rangeland management techniques in Abu Dhabi. J. Arid Environ. 35, 95–110.

Osman, A.E., Makawi, M., Ahmed, R., 2008. Potential of the indigenous desert grasses of the Arabian Peninsula for forage production in a water scarce region. Grass Forage Sci. 63, 495–503.

Peakall, R., Smouse, P.E., 2012. GenAlEx 65: genetic analysis in excel population genetic software for teaching and research—an update. Bioinformatics 28, 2537–2539.

Ranamukhaarachchi, D.G., Kane, M.E., Guy, C.L., Li, Q.B., 2000. Modified AFLP technique for rapid genetic characterization in plants. Biotechniques 29, 858–866.

Sinha, R.K., Bhatia, S., Vishnoi, R., 2009. Desertification control and rangeland management in the Thar desert of India. In: Rala, Report no. 1996. 200, pp. 115–123.

Subudhi, P.K., Parami, N.P., Harrison, S.A., Materne, M.D., Murphy, J.P., Nash, D., 2005. An AFLP-based survey of genetic diversity among accessions of sea oats *Uniola paniculata*, Poaceae from the southeastern Atlantic and Gulf coast states of the United States. Theor. Appl. Genet. 111, 1632–1641.

Todd, J., Wu, Y.Q., Wang, Z., Samuels, T., 2011. Genetic diversity in tetraploid switchgrass revealed by AFLP marker polymorphisms. Genet. Mol. Res. 104, 2976–2986.

Travis, S.E., Maschinski, J., Keim, P., 1996. An analysis of genetic variation in *Astragalus cremnophylax* var c*remnophylax*, a critically endangered plant, using AFLP markers. Mol. Ecol. 5, 735–745.

Ubi, G.N., Kenworthy, W.J., Costa, J.M., Cregan, P.B., Alvernaz, J., 2003. Genetic diversity of soybean cultivars from China Japan, North America, and North American ancestral lines determined by amplified fragment length polymorphism. Crop Sci. 43, 1858–1867.

Vaiman, D., Mercier, D., Moazami-Goudarzi, K., Eggen, A., Ciampolini, R., Lepingle, A., Velmala, R., Kaukinen, J., Varvio, S.L., Martin, P., Leveziel, H., Guerin, G., 1994. A set of 99 cattle microsatellites, characterization, synteny mapping and polymorphism. Mamm. Genome 5, 288–297.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van Der Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23, 4407–4414.

Xie, W., Zhang, X., Cai, H., Liu, W., Peng, Y., 2010. Genetic diversity analysis and transferability of cereal EST-SSR markers to orchardgrass *Dactylis glomerata* L. Biochem. Syst. Ecol. 38, 740–749.

Young, A.G., Brown, A.H.D., Murray, B.G., Thrall, P.H., Miller, C.H., 2000. Genetic erosion, restricted mating and reduced viability in fragmented populations of the endangered grassland herb *Rutidosis leptorrhynchoides*. In: Young, A.G., Clarke, G.M. (Eds.), Genetics, Demography and Viability of Fragmented Populations. Cambridge University Press, Cambridge, pp. 335–359.

Zalapa, J.E., Price, D.L., Kaeppler, S.M., Tobias, C.M., Okada, M., Casler, M.D., 2010. Hierarchical classification of switchgrass genotypes using SSR and chloroplast sequences: ecotypes, policies, gene pools, and cultivars. Theor. Appl. Genet. 122, 805–817.