FINGERPRINTING OF SOME OMANI DATE PALM CULTIVARS USING SSR MARKERS

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The Sixth International Date Palm Conference (SIDPC)
Abu Dhabi -UAE: 19 – 21 March, 2018.

Abstract: The numbers of known date palm cultivars that are distributed all over the world are approximately 5,000, out of which about 250 are found only in Oman. It is a dioecious, perennial, monocot plant, and its heterozygous form makes its progeny strongly heterogeneous. Most of these cultivars were described using morphological markers such as fruit and vegetative traits, but these are greatly affected by the environment and are also complex. In general, the identification and evaluation of genetic diversity between the cultivars on the basis of morphological markers is difficult. Recently DNA markers have been used to provide the information on the relatedness of date palm cultivars that are difficult to distinguish morphologically. Microsatellites (SSR) have been used widely in date palm genetic diversity among GCC countries. In this study, the genetic diversity of twelve of Omani date palm cultivars was studied by using 10 microsatellite markers which is same markers used in GCC countries date palm cultivars. Mature leaves samples of all cultivars, five replicates per cultivar, were collected from five governorates of Oman from north and south Al-Batinah (Sohar and Barka), Al-Dahirah (Ibri), Al-Dakhilia (Wadi Quriate, Nizwa), Al-Sharqiya (Samad Al-Shan, Al-Kamel & Al-Alwafi) and Al-Burymi (Mahadah, Al-Buraymi). A total of 113 alleles were scored with average of 11.3 alleles per locus. It was ranged from 5 alleles/locus for SSR (PDCAT 17) to 17 alleles/locus SSR (mPdCIR 10). The polymorphic information content (PIC) average 0.668. There was genetic diversity within and among the selected cultivars and were assessed by using microsatellite markers.

INTRODUCTION

Microsatellite or simple sequence repeat (SSR) molecular markers have been proven to be very powerful in plant diversity analysis because they are locus specific, co-dominant, highly polymorphic and highly reproducible. Genetic variation in the date palm germplasm has been traditionally characterized using morphological descriptors. However, such morphological markers are often unreliable and ambiguous because of the influence of environmental factors and confounding effects of developmental stage of the plant) Barrow, 1998). The aim of this investigation was to study the genetic variation (polymorphisms) among different Omani date

palm cultivars using SSR markers. Date palm (*Phoenix dactylifera* L.) is a dioecious perennial monocotyledon plant with long generation times (a period of 4 to 5 years is necessary to reach the first flowering) that belongs to *Arecaceae* family (Elhoumaizi, 2002). It is important to study the genetic diversity of the Omani date palm cultivars, because it helps to find the identity of DNA that will helps in documenting the Omani cultivars in order to preserve them. Also, to determine the strains within species, make sure and check conventional seedlings before being placed in the tissue propagation, definition promising new cultivars for farmers, to make sure the genetic stability of the output of agriculture and textile version genetic compatibility with the original certification and to ensure product quality.

The most common characteristics that are used to identify different cultivars of date palm are the morphology of leaves, spines, and fruit, which are mainly based on the characterization of introduced date palm cultivars in California (Nixon, 1950). Over the years, many date palm cultivars have been transplanted to areas other than the area of their origin, and they may have been given different names. As a result, a variety may have different names in different areas, or two genetically different varieties may have the same name. This may reduce the genetic diversity of the cultivars, making them vulnerable to biotic and abiotic stresses. In general, the identification and evaluation of genetic diversity between cultivars on the basis of morphological markers is difficult. The identification of trees is usually not possible until the onset of fruiting, which takes 3 to 5 years. Further, characterizing varieties requires a large set of phenotypic data that are difficult to access statistically and are variable because of environmental effects (Sedra *et. al.*, 1993, 1996, 1998). Biochemical markers (isozymes and proteins) are effective in varietal identification (Bennaceur *et. al.*, 1991; Fakir et al., 1992; Bendiab *et. al.*, 1993). However, they give limited information and are an indirect approach for detecting genomic variation (Elmeer, 2015).

In this study, we aimed to investigate the genetic diversity of date palms in Oman to improve production and reveal the genetic relationships among 10 date palm cultivars using 12 nuclear microsatellite markers. These cultivars have names that were given by farmers after continuous selection.

MATERIAL AND METHODS

Collection of material

Date palm material was collected 5 replicate of 12 cultivars from the different region on Oman (North and South Al-Batinah governorate, Al-Dahrah, Al-Dakhliah, Al-Sharqeah, Al-Buraimi) as shown in Table 1 and Fig. 1. These cultivars represent the diversity of date palm genotypes in the Omani date palm plantation. Young leaves from mature, randomly sampled trees, were collected and stored at -80°C, until DNA extraction.

Molecular analysis

DNA was extracted by using (Maxi: kit Qiagen Cat # 68163 DNeasy plant). It was measured DNA concentration using a 1% of Agarose gel and then detected using UV light and was used the device (Nanodrope) to ensure the purity of DNA by absorption measurement at a wavelength between 260 and 280 nm to enter it in The next stage figure2. The microsatellite amplification reaction was performed by using Applied Biosystems (2720 thermo cycler, Singapore) with 10 primers combinations. Table 2 shows the microsatellite combination and its allelic ranges (Peakall 2012). The PCR program had initial denaturation at 95 ^{0}C for 5 minutes, then 35 cycles

of 95 ^{0}C for 30 minutes, 52/55 ^{0}C for 1 min, and 72 ^{0}C for 1 minute and final elongation step at 72 ^{0}C for 7 minutes. Amplification products were separated using 2% agarose gel electrophoresis shown figure3. The microsatellite alleles were detected using Beckman coulter CEQTM 8000 automated DNA sequencer machine. Control sample in replication was used in this experiment along with the samples to be analyzed, to ensure the repeatability and accuracy of results.

RESULTS AND DISCUSSION

The targeted fragments and allele scoring were performed by fragment analysis. For each marker, the average number of alleles per locus, the expected heterozygosity (He) and the observed heterozygosity (Ho) were calculated by Gene Alex 6.3 software .The genetic similarity and the analysis of molecular variance (AMOVA) and principal coordinates analysis (PCoA) were also calculated using Gene Alex 6.3 software. DARwin 6.0 software was used to make dendrogram which showed the distribution of different individuals.

A total of 113 alleles were scored with average of 11.3 alleles per locus. It was ranged from 5 alleles/locus for SSR (mPdCIR 57) to 20 for locuse SSR (mPdCIR 10). The polymorphic information content (PIC) average .6650 and it was range between 0.4818 (PDCAT21) and 0.9125 (mPdCIR 10) (Table 3). The average of expected heterozygosity (He) ranged between 0.227 (mPdCIR 85) and 0.718 (mPdCIR 10) and the average of observed heterozygosity (Ho) ranged between 0.150 (mPdCIR 85) and 0.700 (mPdCIR 16) (Table 3). Most of markers, the observed heterozygosity value was higher than the expected one. The percentage of polymorphic loci per cultivar varied between 60% and 100% with an average of 85% (Table 4). Molecular variance analysis showed that 70 % of the variation was due to differences within populations, while 30 % was due to differences between populations in Omani cultivars (Fig. 4).

The Dendrogram shown in figure 5, illustrates the divergence between the studied Omani date palm cultivars and suggests their tree brunching, Dendrogram divided into four main groups, Group A has the following cultivars (Lolo, Menaz, Kash Qantrah, Shahel, Hilali Makran). While Group B has the following cultivars (Selani, Hasas, Manhe) and group C has the following cultivars (Nasho Al-khashba, Hilali Asfer, Merzaban, Hilali A-Hasa) and group D has combination with different following cultivars: Hilali Alhasa, Shahel, Merzaban (Fig. 6). The principal coordinates analysis (PCoA) of the 12 Omani cultivars showed that the majority of cultivars were grouped in cluster and also dispersed among different sub-clusters. DNA SSR markers are powerful tool to provide information on the relatedness of varieties that are difficult to distinguish morphologically, thus helping in the management of plant accessions and in breeding programs. In this study, SSR markers have been used to assess the molecular characterization and the phylogenic relationships of Omani date palm cultivars. Present results provide evidence of a genetic diversity

CONCLUSION

This study showed the distribution of Omani date palm cultivars from different region in Oman and analysis based on SSR markers. In future s this technique will help our study and provide a useful tool for research on genetic diversity, gene mapping, and marker-assisted selection in date palm. Therefore, while allowing studies on genetic variation, SSR markers also provides information on gene function related to possible phenotypic differences between the date palm cultivars.

References

-Barrow S. 1998. A monograph of Phoenix L. (*Palmae: Coryphoideae*). Kew Bull 53: 513–575.

-Bendiab K., M. Baaziz, Z. Brakez and M.H. Sedra. 1993. Correlation of isoenzyme polymorphism and Bayouddisease resistance in date palm cultivars and progeny. *Euphytica* 65:23-32

-Bennaceur M., C. Lanaud, M.H. Chevalier and N. Bounagua. 1991. Genetic diversity of date palm (Phoenix dactylifera L.) from Algeria revealed by enzyme markers. *Plant breed* 107:56-69.

-Elhoumaizi M.A., M. Saaidi, A. Oihabi and C. Cilas. 2002. Phenotypic diversity of date-palm cultivars (*Phoenix dactylifera* L.) from Morocco. *Genet. Resour. Crop. Evol*. 2002; 49: 483–490. doi: 10.1023/A:1020968513494.

-Elmeer K. and I. Mattat. 2015. Genetic diversity of Qatari date palm using SSR markers. *Genetics and Molecular Research*, 14(1), pp.1624-1635.

-Fakir S. and P. Munier. 1981. Origine de la culture sur palmier dattier et sa propagation en Afrique. *Fruits* 36 (437-450).

-Nixon R.W. (1950). Imported Cultivars of Dates in the United States. United States Department of Agriculture Circular No. 834, Washington, D.C.

-Peakall R. and P.E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics* 28: 2537-2539.

-Sedra M.H., H. Filali and D. Frira. 1993. Observations sur quelques caracteristiques phenotypiques et agronomiques du fruit des varieties et clones du palmier dattier selectionnes. *Al Awamia* 82: 105-120.

-Sedra M.H., H. Fila, A. Benzine and M. Allaoui. 1996. La palmeraie dattière marocaine : évaluation du patrimoine phenicicole. *Fruits* 1: 247-259.

-Sedra M.H., Lashermes P., Trouslot P. and M.C. Combes. 1998. Identification and genetic diversity analysis of date palm (Phoenix dactylifera L.) varieties from Morocco using RAPD markers. *Euphytic*a 103: 75-82.

No	Locus Name	Sequences (5 '-3')	Annealing T_m (^{0}).	Motif repeat	Expected Allelic range(bp)	Observed Allelic range(bp)

Tables

Table 1. Samples used in fingerprint study

No	Sample Name	Code	Location	No. replicate
1	Selani	SL	Batinah,Al-Dahrah,Al-Dakhliah,al-sharqeah,Buraimi	5
2	Hasas	HSS		
3	Merzaban	MR		
4	Shahel	SHL		
5	Menaz	MNZ		
6	Manhe	MNH		
7	Lolo	LO		
8	Hilali Makran	HM		
9	Hilali Alhasa	HH		
10	Hilali Asfer	HA		
11	Nasho alkhashba	NK		
12	Khash Qantrah	KK		

1	PDCAT10	F: CACTGCTCCTGTTGCCCTGT R: TGTAGAAGGGCAGAGGACGG	55°C	$(TC)_{16}$	107-127	114-128
2	PDCAT14	F: TGCTGCAAATCTAGGTCACGA R: GTTTACCCCTCGGCCAAATGTAA	55°C	$(TC)_{19}(TC)_{16}$	101-155	135-168
3	PDCAT17	F: CAGCGGAGGGTGGGCCTC R: GTTTCTCCATCTCCCTTTTTCTGCTACTC	55°C	$(GA)_{21}$	116-145	143-165
4	PDCAT20	F: TTTCAGACACATCAAGTAACGATGA R: GTTTACGTCCACCCCAAGTTACGA	55°C	$(GA)_{29}$	294-353	343-361
5	PDCAT21	F: GTGTTTGAAGATTGATTTTGTGTTATGAG R: GTTTCGAACTATGCACAATAGTATATTG	55°C	$(GA)_5T(GA)_2TA(GA)_2$ $GC(GA)_5(GT)_7$	144-150	143-163
6	mPdCIR 10	F: ACC CCG GAC GTG AGG TG R: CGT CGA TCT CCT CCT TTG TCT C	52°C	$(GA)_{22}$	118-161	130-152
7	mPdCIR 15	F: AGC TGG CTC CTC CCT TCT TA R: GCT CGG TTG GAC TTG TTC T	52°C	$(GA)_{15}$	120-156	140-157
8	mPdCIR 16	F: AGC GGG AAA TGA AAA GGT AT R: ATG AAA ACG TGC CAA ATG TC	52°C	$(GA)_{14}$	130-138	143-157
9	mPdCIR 85	F: GAG AGA GGG TGG TGT TAT T R: TTC ATC CAG AAC CAC AGT A	52°C	$(GA)_{29}$	152-183	174-200
10	mPdCIR 93	F: CCA TTT ATC ATT CCC TCT CTT G R: CTT GGT AGC TGC GTT TCT TG	52°C	$(GA)_{16}$	153-184	160-181

Table 2. List of microsatellite primers designed for date palm by Billotte el al. (2004) and Akkak et al. (2009), marker name, annealing temperature Tm (^{0}), motif repeat, observed allelic size range (bp) and status of amplification.

Table 3. The PIC values and alleles/loci of 17 microsatellite primer combination and Heterozygoty of Omani cultivars calculated with GenAlex 6.3 software He: average of expected heterozygosity; Ho: average of observed heterozygosity.

Locus Name	Alleles/loci	PIC Value	He	Ho
PDCAT10	10	0.639	0.627	0.683
PDCAT14	10	0.705	0.262	0.167
PDCAT17	5	0.573	0.375	0.483
PDCAT20	9	0.555	0.427	0.483
PDCAT21	7	0.548	0.420	0.517
mPdCIR 10	17	0.916	0.718	0.450
mPdCIR 15	15	0.879	0.672	0.683

mPdCIR 16	13	0.779	0.610	0.700
mPdCIR 85	11	0.570	0.227	0.150
mPdCIR 93	16	0.798	0.682	0.650
Total	113	-		
Average	11.3	0.668		

Table 4. Percentage of polymorphic loci of Omani cultivars

Population	Percentage (%)
Selani	80
Hasas	70
Merzaban	90
Shahel	100
Menaz	90
Manhe	100
Lolo	60
Hilali Makran	90
Hilali Alhasa	80
Hilali Asfer	90
Nasho alkhashba	80
Kash Qantrah	90
mean	85

Figures

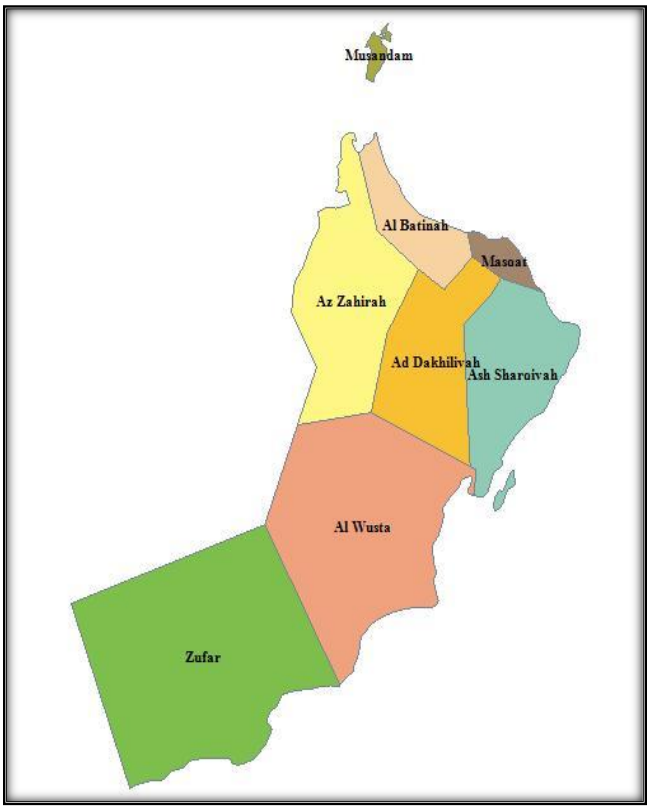

Fig. 1. Locations of Date palm collected

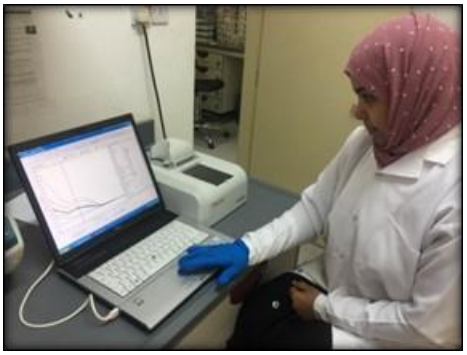

Fig. 2. Measuring the purity of the DNA using a device (Nanodrop).

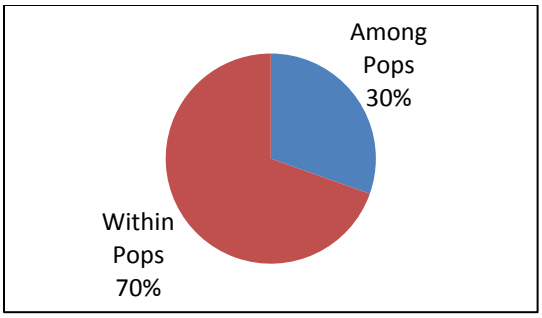

Fig. 3. Analysis of molecular variance between Oman cultivars.

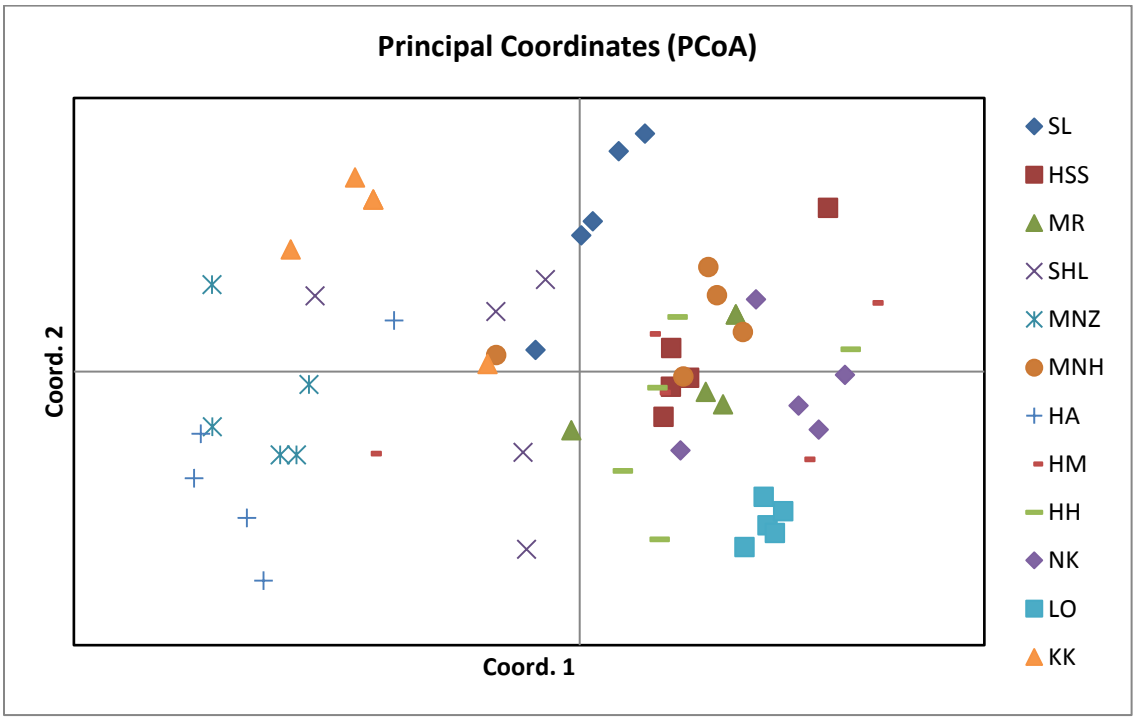

Fig. 4. principal coordinates analysis of Omani date palm cultivars

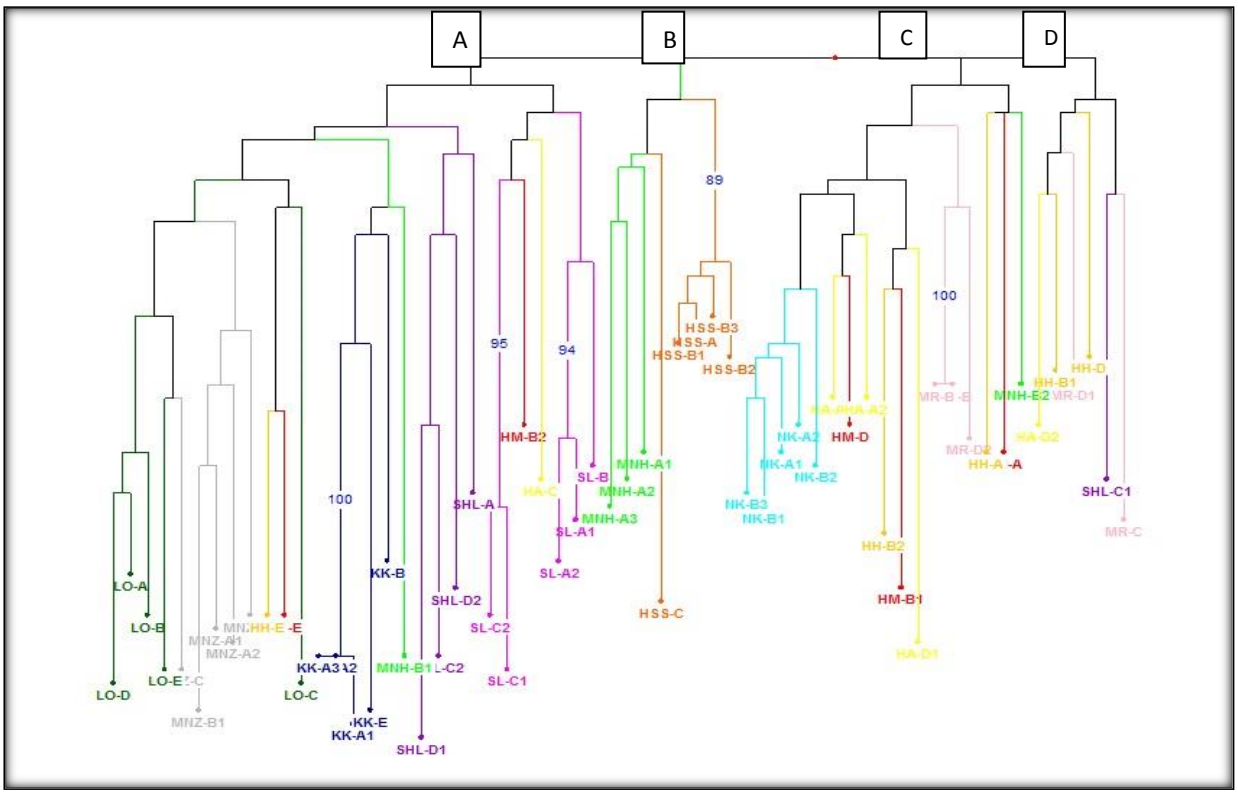

Fig. 5. Dendrogram of similarity coefficients based on UPGMA cluster analysis of 12 genotypes using 10 microsatellite (SSR) primer pairs**.**

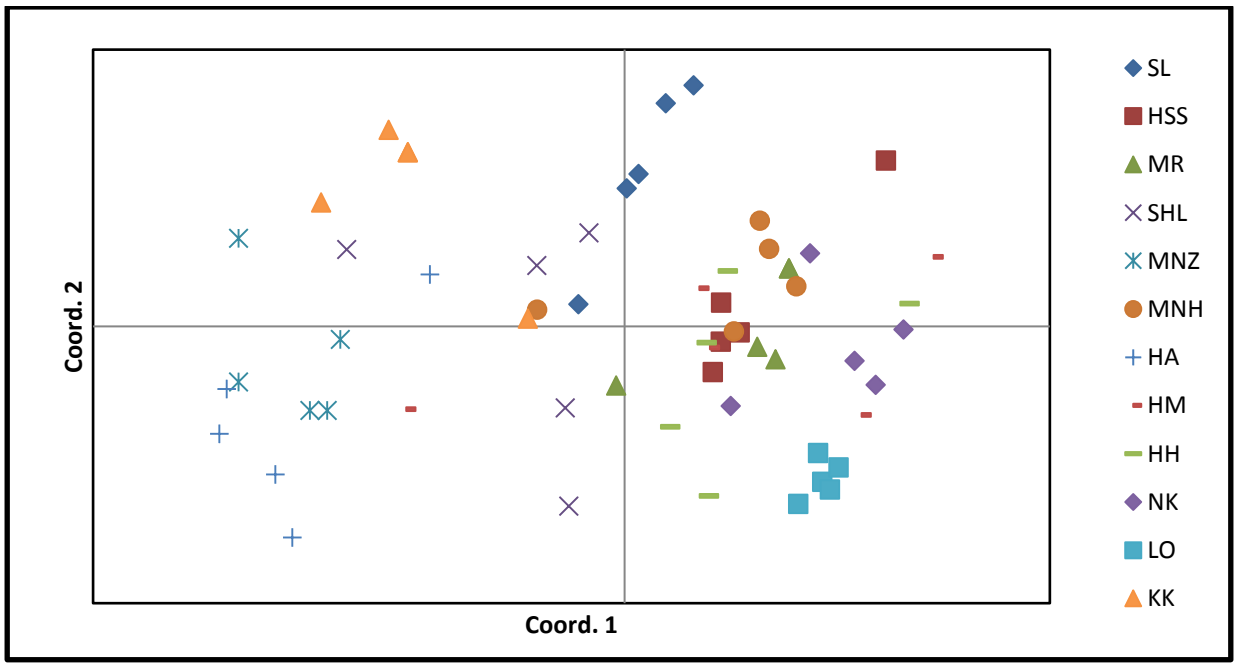

Fig. 6. Principal Coordinates Analysis (PCoA) of 12 Omani date palm cultivars