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MORPHOLOGICAL CHARACTERIZATION OF PAKISTANI DATE PALM (*Phoenix dactylifera* L.) GENOTYPES

Summar A. Naqvi[1,*], Iqrar A. Khan[1], Jean Christophe Pintaud[2], M. Jafar Jaskani[1] and Asif Ali[3]

[1]Institute of Horticultural Sciences, University of Agriculture, Faisalabad-38040, Pakistan; [2]IRD, DIADE-DYNADIV, Laboratoire Genetrop, 911 Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France; [3]Department of Plant Breeding & Genetics, University of Agriculture, Faisalabad-38040, Pakistan
[*]Corresponding author's e-mail: summarnaqvi@hotmail.com

Twenty Pakistani date palm cultivars from nine different geographical regions were characterized to estimate the polymorphism and possible similarity level. Nineteen morphological traits were explored and subjected to principal component analysis (PCA), cluster analysis (CA) and correlation index. The results brought out important differences in phenotypic characters in all date palm cultivars. Principal component analysis (PCA) indicated that all quantitative traits along with number of wings, frequency of wings, seed surface and seed shape were highly divergent. Similarly cluster analyses (CA) also revealed morphological variability among date palm cultivars and, some relationship and heterogeneity was also observed within cultivars of the same origin.
Keywords: Date palm, phenotypic characters, polymorphism, cluster analysis

INTRODUCTION

The date palm (2n=36), (*Phoenix dactylifera* L.), a dicotyledonous and perennial plant, belongs to genus *Phoenix* and family *Arecaceae*, is a very important commercial crop of warm arid and semi-arid region of South and West Asia and North Africa (Moore, 1963; Bailey and Bailey, 1976; Dowson, 1982). The fruit is a rich source of carbohydrates, constituting mainly glucose and fructose, thus providing cheap nutrition to the millions of individuals worldwide and considered alternate to food security (Al-Farsi *et al*., 2007; Rastegar *et al*., 2012; Abul-Soad *et al*., 2013; Haider *et al*., 2013). *Phoenix dactylifera* originates from Arabian Gulf and North Africa (Nixon, 1951; Wringley, 1995) and currently, it is cultivated in the Middle East, North Africa, parts of Central and South America, Southern Europe, India and Pakistan (Dowson, 1982; Zaid, 1999). Approximately 14 Species have been categorized within the *Phoeix* genera and family Arecaceae has more than 183 genera and 2600 species (Dransfield, 2008).

Botanically, date palm is the giant cylindrical *Phoenix* specie can grow up to 30 m taller (Barrow, 1998) with constant diameter not more than *P. canariensis* specie. It is a single trunked (unbranched) and contains dead leaf bases (Nixon, 1951). The mature date palm produce 100-120 number of green leaves (3-5 m). Each leaf contains varying numbers (120-150) of leathery, fleshy leaflets with acute tips on both sides; whereas, petiole contains a number (20-40) of spines which later on modifies into the basal leaflets. Both leaflets and spines vary in their length depending on their position of leaves, cultivar type and climatic conditions. Due to dioecious nature the pistillate and staminate flowers are borne in clusters on tapering rachis and enclosed in spathe when immature on separate plants. Male spathe was generally higher in diameter than female spathe and rapture longitudinally at the time of anthesis (Uhl and Dransfield, 1987). Flowers are fleshy and creamy yellow if male; whereas, female flowers are yellow in color with nice and sweet odor (Chao and Krueger, 2007). Date palm produces aerial and basal suckers which are further used for propagation in order to obtain true to type progeny (Al-Yahyai and Manickavasagan, 2012).

The characterization (genetical, morphological and biochemical) of trees is very important in terms of providing useful information on its definite/efficient use (Ahmed *et al*., 2011; Akhtar *et al*., 2014; Mehmood, 2015). Date fruit is highly nutritious with excellent taste and flavor, is the basic part of the human diet (Vayalil, 2002). The shelf-life of dates can be enhanced by drying traditionally (sun-drying) or placing in low-temperature storage. Chemically, date fruit is composed of a high percentage of carbohydrates (glucose and fructose), proteins, vitamins, fats, dietary fibers, minerals and low amount of starch depending on cultivar type and fruit developmental stage (Vayalil, 2011).

Egypt is the world largest producer of dates, followed by Saudi Arabia, Iran, UAE and Pakistan. The date industry is well adopted in Pakistan with an annual production of more than 72 thousand tonnes (FAO, 2013) and contributing 10.3 % in total world production. The major date palm growing areas are plains and semi-desert like zones in Sindh, oasis eco-system and river valleys in Balochistan, Irrigated plains and Suleman mountain pediments in Punjab and some parts of Khyber Pakhtukhwa (KPK), Pakistan (Haider *et al*., 2013). Dhakki, Aseel, Makran, Begum Jungi, Gulistan, and Sabzo

are amongst the elite indigenous cultivars and numerous other local date cultivars are grown in other parts of the country (Markhand *et al*., 2010; Iqbal *et al*., 2011). Some local cultivars have been denominated by the owner/farmers on the basis of their fruit color, sweetness, presence at the specific location and morphological parameters (Ahmed *et al*., 2011); misnaming of homonymous and synonymous trees is the main problem of Pakistan date industry. This flourished industry is at threat of various insects (red palm weevil, giant palm weevil and dubus bug) and diseases (sudden death).

Accurate characterization of date palm cultivars is the strategy for the establishment of commercial orchards and registered nurseries that can guarantee uniformity in germplasm. Breeders focus on the selection of genetically diverse parents for hybridization process, as the higher range of variability in parents leads to more paths in improving the target traits (Pommer, 2012). So, the information about genetic variation and its linkage between populations is important to better understand the available genetic inconsistency for further use in potential breeding programs. This characterization needs huge set of phenotypic (qualitative and quantitative) data (Rao, 2004), that provide basis for an assessment of genetic diversity among date palm germplasm (Salem *et al*., 2008; Hammadi *et al*., 2009; Ahmed *et al*., 2011). However, our studies provide first insight into the phenotypic variation in different date palm accessions of diverse geographical origin.

MATERIALS AND METHODS

***Plant material*:** Seeds of 20 different date palm accessions were collected from 9 different geographical regions of Pakistan (Jhang, Layyah, Muzafargarh, Bahwalpur, Rahim Yar Khan, Sukkur, Khairpur, Kech and Gwadar) for morphological characterization. Details of the accessions along with their coordinates are given in Table 1.

***Methodology*:** Nineteen traits (5 quantitative and 14 qualitative) were measured on 600 date palm seeds representing 20 date palm accessions i.e. 30 seeds/accessions. Each seed of each accession (out of the 30 seeds) was assigned a number 1-30. Morphometric traits were observed visually using standard descriptors of the date palm (IPGRI, 2005).

***Statistical analysis*:** Multivariate analysis was done using XLSTAT version 3.06. PCA and CA were executed for the measurement of correlation and the plot projections of variables and accessions as well (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

***Principal component analysis, correlation of morphological variables and cluster analysis*:** The principal component analysis distributed 19 morphological variables of seeds in six components explained 85.245% of cumulative diversity (Table 3). The first factor accounted 24.387% of the total variation comprising on base to apex and embryo to apex length, dorsal and lateral area and ventral furrow nature. In case of factor 2, 18.199% variation was observed on the basis of embryo to apex length/base to apex length ratio, seed wings, frequency and shape. Moreover, 4th, 5th and 6th factor showed 13.101, 9.717 and 5.190% variability, with highest factors load in seed wings

Table 1. Details of the 20 different date palm accessions studied

Sr.	Accessions ID	Accession	District	Collection Site	Latitude	Longitude	Elevation
1	DJP	Dhakki	Jhang	Date palm Research Station	31, 15.557	72, 19.960	492
2	AJP	Aseel	Jhang				
3	HJP	Hillawi	Jhang				
4	KJP	Khadrawi	Jhang				
5	ZJP	Zehdi	Jhang				
6	SJP	Seib	Jhang				
7	KJP	Kozan Abad	Jhang				
8	RJP	Rachna	Jhang				
9	LLP	Lonri	Layyah	Distirct Government Orchard	30, 57.212	70, 55.962	475
10	MMP	Murkran	Muzafargarh	Khudai Farm	30, 23.688	71, 23.961	396
11	HBP	Halwain	Bahawalpur	Horticulture Research Station	29, 22.796	71, 38.787	335
12	TBP	Taar wali	Bahawalpur				
13	GBP	Gul-e-Lal	Bahawalpur				
14	ORYKP	Ood kafir	Rahim Yar Khan	Arain wala bagh	28, 55.765	70, 40.227	336
15	PRYKP	Pir wali	Rahim Yar Khan	Basti Syed Malok Shah	28, 55.795	70, 40.224	276
16	LSS	Lasora	Sukkur	Got Muhammad Bachal, U.C. Babirlo	27, 38.908	68, 49.195	194
17	DKS	Dokar	Khairpur	Date Palm Research Station	27, 20.095	68, 40.799	193
18	BKS	Begum Jungi	Khairpur				
19	LKB	Lolo	Kech	Mairaj Muhammad Khan orchard	26, 01.956	63, 19.474	804
20	SGB	Surkh	Gwadar	Jiwani	25, 05.211	61, 46.554	4

and their frequency (4th), seed surface, micropyle elevation and ventral furrow ends (5th); and ventral furrow width and seed base (6th). Whereas, micropyle appearance was observed in seeds of all 20 date palm accessions (Table 3). Genetic variation among all the accessions could be assessed in 2D principal component analysis (PCA) plot, the first two factors are shown in the Fig.1. The accessions are scattered in all 4 planes. Accessions close to central axis are less diverse while accessions away from the central axis showed highest variability like SGB from Gwadar-Balochistan, HBP and GBP from Bahawalpur-Punjab. SGB and GBP are highly diverse and differentiated from each other on the basis of base to apex length, embryo to apex length, dorsal area, lateral area, seed shape and seed apex while HBP was deviated having high embryo to apex length/base to apex length ratio, seed wings and frequency of wings. Correlation among morphological variables, EAL, DA and LA were highly significant ($p>0.05$) and positively correlated with each other while in case of qualitative variables, MPO was highly significant and correlated to EAL. MEL was the only variable differentiated from almost all the variables except EAL, SSH, SCO, VFN and MPO.

Date palm accessions were successfully clustered into three different classes according to the phylogenetic distance (Fig.2). All the accessions were placed in between the dissimilarity level from 1 to 301. Main class (1) was a big

Table 2. Morphological traits of date palm seeds

Characters	Denominations	Unit
Base to Apex Length	BAL	(cm)
Embryo to Apex Length	EAL	(cm)
Dorsal Area	DA	(cm^2)
Lateral Area	LA	(cm^2)
Embryo to Apex Length/Base to Apex Length	EAL/BAL	(%)
Seed shape	SSH	
Seed color	SCO	
Seed surface	SSU	
Seed base	SBA	
Seed apex	SAP	
Seed wings	SWI	
Frequency of wings	FOW	
Ventral furrow shape	VFS	
Ventral furrow width	VFW	
Ventral furrow ends	VFE	
Ventral furrow nature	VFN	
Micropyle position	MPO	
Micropyle elevation	MEL	
Micropyle appearance	MAP	

Table 3. Correlation coefficient among morphological (quantitative and qualitative) traits of 20 Pakistani date palm accessions representing 10 locations

Variable	BAL	EAL	EL/BL	DA	LA	SSH	SCO	SSU	SBA	SAP	SWI	FOW	VFS	VFW	VFE	VFN	MPO	MEL	MAP
BAL(cm)	**1.000**																		
EAL(cm)	0.841	**1.000**																	
EL/BL%	0.166	0.669	**1.000**																
DA (cm^2)	0.810	0.683	0.150	**1.000**															
LA cm^2)	0.805	0.605	0.024	0.967	**1.000**														
SSH	-0.388	-0.094	0.387	-0.220	-0.257	**1.000**													
SCO	-0.342	-0.209	0.078	-0.238	-0.270	0.644	**1.000**												
SSU	0.057	0.179	0.234	-0.106	-0.119	0.251	0.301	**1.000**											
SBA	-0.033	0.080	0.185	0.247	0.187	0.165	0.177	-0.395	**1.000**										
SAP	-0.312	-0.330	-0.189	-0.552	-0.511	-0.023	0.009	0.430	-0.435	**1.000**									
SWI	-0.178	0.030	0.361	-0.057	-0.072	0.232	-0.146	-0.132	0.178	-0.186	**1.000**								
FOW	-0.178	0.030	0.361	-0.057	-0.072	0.232	-0.146	-0.132	0.178	-0.186	1.000	**1.000**							
VFS	-0.159	-0.182	-0.160	0.252	0.230	0.100	0.296	-0.132	0.317	-0.285	-0.404	-0.404	**1.000**						
VFW	0.006	0.062	0.082	-0.156	-0.265	-0.027	0.084	0.036	0.055	0.419	-0.391	-0.391	-0.198	**1.000**					
VFE	-0.060	-0.071	-0.033	-0.200	-0.134	-0.337	-0.366	-0.192	-0.258	0.270	0.076	0.076	-0.178	0.147	**1.000**				
VFN	0.665	0.425	-0.150	0.400	0.453	-0.425	-0.381	-0.081	-0.070	-0.295	0.096	0.096	-0.332	-0.345	-0.140	**1.000**			
MPO	-0.061	-0.505	-0.822	0.080	0.231	-0.314	-0.134	-0.216	-0.044	0.085	-0.360	-0.360	0.211	-0.033	0.025	0.031	**1.000**		
MEL	-0.026	-0.236	-0.371	-0.010	0.079	0.362	0.260	0.000	-0.068	-0.162	-0.115	-0.115	0.076	-0.379	-0.250	0.210	0.336	**1.000**	
MAP																			

group including 10 accessions while class (2 and 3) contains 4 and 6 accessions respectively. Among these 20 genotypes collected from 9 different geographical locations, there was arrangement according to the sampling locality. For example, genotypes from Jhang (AJP, RJP, HJP, DJP, KJP, and SJP) grouped together in class 3 and a nearby class 2. Class 1 possessed genotypes (BKS, DKS, PRYKP, GBP, ORYKP, TBP) representing Bahwalpur, Rahim Yar Khan and Khair pur.

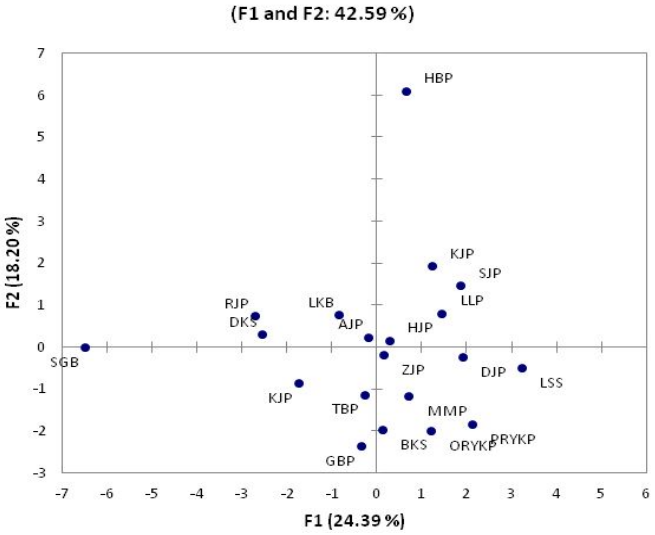

Figure 1. Principal component analysis plot representing morphological diversity based on the component 1 & 2 of 20 Pakistani date palm accessions

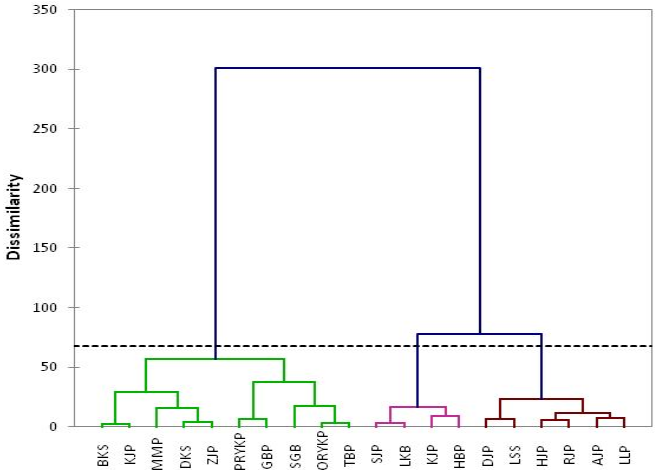

Figure 2. Dendrogram of 20 Pakistani date palm accessions discriminating accessions into their actual group.

Previously, many authors have considered different parts of date palm tree as suitable variables for the estimation of morphological diversity (Hammadi *et al*., 2009; Ahmed *et al*., 2011). However, we used seed as a considerable diversity estimation tool. Earlier, Elshibli and Korpelainen (2009) and Ahmed *et al*. (2011) worked only on few quantitative seed traits.

Our results indicated huge diversity in selected date palm genotypes. It is proved from projection of principal component analysis plot and AHC cluster analysis. Seed length, seed width, dorsal area, lateral area, number of wings, frequency of wings, seed surface and seed shape had a big

Table 4. First 6 components of PCA analysis of 19 morphological (quantitative & qualitative) characters in 20 Pakistani date palm accessions

Characters	F1	F2	F3	F4	F5	F6
Base to apex length	**0.899**	-0.139	-0.199	-0.266	0.163	0.096
Embryo to apex length	**0.801**	0.324	-0.152	-0.456	0.038	0.011
Embryo to apex length/Base to apex length	0.238	**0.815**	-0.002	-0.421	-0.137	-0.112
Dorsal area	**0.904**	-0.135	0.199	-0.129	-0.091	-0.108
Lateral area	**0.888**	-0.237	0.180	-0.021	-0.008	-0.151
Seed shape	-0.358	**0.490**	**0.597**	-0.213	0.216	0.041
Seed color	-0.417	0.122	**0.628**	-0.386	0.145	0.090
Seed surface	-0.160	0.154	-0.093	-0.571	**0.589**	-0.253
Seed base	0.196	0.183	**0.525**	0.085	-0.568	**0.369**
Seed apex	-0.606	-0.117	-0.528	-0.246	0.188	-0.002
Seed wings	0.072	**0.772**	-0.029	**0.578**	0.038	-0.018
Frequency of wings	0.072	**0.772**	-0.029	**0.578**	0.038	-0.018
Ventral furrow shape	-0.008	-0.375	**0.638**	-0.145	-0.373	-0.463
Ventral furrow width	-0.253	-0.101	-0.347	-0.569	-0.388	**0.480**
Ventral furrow ends	-0.128	-0.029	-0.609	0.197	-0.298	-0.346
Ventral furrow nature	**0.668**	-0.112	-0.188	0.272	**0.434**	0.283
Micropyle position	-0.082	-0.809	0.087	0.339	0.017	0.057
Micropyle elevation	-0.050	-0.279	**0.496**	0.237	**0.592**	0.139
Micropyle appearance	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Eigenvalue	4.390	3.276	2.637	2.358	1.749	0.934
Variability (%)	24.387	18.199	14.651	13.101	9.717	5.190
Cumulative %	24.387	42.586	57.237	70.338	80.055	85.245

share in variability. Earlier, Elhoumaizi *et al*. (2002) and Ahmad *et al*. (2011) reported wide range of morphological diversity within genotypes. The topography of the dendrogram of 20 Pakistani date palm accessions discriminating accessions into their actual group. In fact, cluster analysis separated all the genotypes in to two major groups; 1) location (Jhang) border area between central and southern Punjab and 2) southernmost Punjab (Bahwalpur, Rahim Yar Khan) and central Sindh (Khairpur) whereas accessions from Balochistan spread in to class 1 and class 2. Few escapes from Jhang were also observed in class 1. It is evident on introduction of elite varieties to other regions.

To sum up the discussion, seed morphological traits proved to be suitable tools to measure the morphological variability among date palm cultivars. However, it needs a large data set to clear about cultivar identification problem. Certainly, a precise description of the morphological and genetic diversity of the Pakistani genetic material requires a combination of biochemical, morphological and molecular markers.

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