EVOLUTIONARY RELATIONSHIP STUDY IN DATE PALM CULTIVARS USING MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS

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Abstract
The present study at S. K. Rajasthan Agricultural University, Bikaner, India on five date palm (*Phoenix dactylifera* L.) cultivars *viz.* Halawy, Khalas, Sewi, Khuneizi and Dayari were assessed for their phylogenetic relationship using morphological and biochemical parameters. The maximum plant height (4.53 m) and number of leaves plant[-1] (29.75) was recorded in cv. Dayari whereas, minimum in cv. Khuneizi (3.56 m). The maximum length (43.48 cm) and breadth of pinnae (3.45 cm) were recorded under the variety of Dayari and Halawy, respectively. The maximum fruit set and length of bunch was recorded in cv. Dayari was 88.50% and 69.00 cm, respectively. The maximum number of bunches (8.75) and fruit drop (47.98%) were recorded in cv. Khalas whereas maximum bunch weight and number of berry strand[-1] was found in cv. Halawy (4.16 kg.) The highest stone weight was observed in Dayari (1.83 g) and maximum pulp-stone ratio was found in cv. Khuneizi (9.57). This evaluation study helped to screen the date palm varieties for better yield. Cultivar Khalas was distantly related to all the investigated cultivars in evolution and construct a separate cluster.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is an important fruit crop which is in cultivation dates back to 4000 BC. The crop is indigenous to countries around Persian Gulf. Date palm is distributed in Iraq, Saudi Arabia, Iran, United Arab Emirates, Oman, Egypt and Algeria, which are the main date palm producing countries in the world (Chandra *et al.*, 1990). The evidence for cultivation of date palm in India has been obtained from excavation of *Mohanjodaro*. However, it is believed that date palm was introduced in India by soldiers of Alexander in the fourth century B C and during Muslim invasion at the beginning of eighth century AD (Haider *et al.*, 2012). The date palm is regarded as a multipurpose palm since every part of the tree has economical value. Value added products such as wine, starch vinegar; arak, jam, chutney, beverages, juice and toffees are prepared from the date fruits. It contains high calorific value (3150 calories kg^{-1} of fresh fruits), 60-65% sugar, and fair amount of fiber (2.5%), protein (2%), fat (2%) and minerals (up to 2%). The date palm leaves are also used for making temporary huts and preparation of baskets, broom, ropes and handicraft items. The seed kernel is used in making cattle feed (Srivashtav *et al.*, 2013). Being tolerant to drought and salinity, it is a potential fruit palm of dry and arid parts of the country (Trifi *et al.*, 2000). Therefore, Rajasthan qualifies to be an ideal home for commercial cultivation of date palm, since majority of agro climatic conditions, such as 25-40°C

temperature for flowering and fruit ripening, accumulation of 3000 degree days heat unit for maturity and rain free condition for fruit ripening required for cultivation of this crop are partly or fully fulfilled in Rajasthan. The varieties like Halawy, Zahidi, Khalas, Barhee and Khadrawy have been found suitable for production of higher yield in Western Rajasthan (Chandra *et al.*, 1992; Singhe *et al.*, 2003).

Since date palm is a cross-pollinated species, a wide genetic diversity has been developed in nature and majority of this has been stabilized, since date palm also propagates vegetative through suckers. All over the world majority of varieties have been developed from this gene pool based on few elite features, which have commercial value. Apart from this, due to selection breeding in this crop, the exact phylogenetic relationship among the cultivars have been lost, which is most required if the deliberate breeding is to be undertaken. Thus, the need of the hour is to have information on cultivar specific and genetic distance among the cultivars. In such situation, where the morphological parameters are not sufficient for varietal identification and assessing phylogeny, the use of biochemical markers has proved to be very handy. Keeping the above view study conducted to compare morphological and biochemical parameters within the varieties to determine the evolutionary distance among the cultivars.

MATERIALS AND METHODS

The experiment was conducted at the Research Farm and Plant Physiology Laboratory of Central Institute for Arid Horticulture (CIAH) and S. K. Rajasthan Agricultural University, Bikaner during 2007 with an altitude of 234.7 m above sea level at latitude 28° 01 N and longitude 73° 22 E. According to Agro Ecological region map brought out by National Bureau of Soil Survey and Land Use Planning, Bikaner falls under Agro ecological region No.2 (M 9 E 1) under arid ecosystem (Hot Arid Eco-region with desert and saline soils), which is characterized by deep, sandy and course loamy, desert soils with low water holding capacity, hot and arid climate, PET (Potential Evapo-transpiration) in this region ranges between 1500-2000 mm. Bikaner has arid climate with an annual average rainfall of about 200-260 mm. The maximum temperature may go as high as 48°C while in the winters it may dip down to 0°C. The palm uniform in size, vigour and bearing stage were selected from 8 years old orchard. Four palm each of the cultivars Halawy, Khalas, Dayari, Khuneizi and Sewi were selected. There were five varieties with four replications planted at 8x8 m distance in square system of planting. In growth parameters, height of plant, girth of plant, spread, number of leaves, length of leaves and size of pinnae were recorded. For the fruiting parameters, fruit set, fruit drop, no. of bunches, and number of strands/bunch etc. was observed during the study. The ripe fruit (turning stage green yellow to golden colour) were harvested for taking the observations and biochemical analyses.

Vegetative characters

Height of the plant (m) - The height of individual tree (m) was measured with the help of long straight bamboo stick and measuring tape. The bamboo stick was placed vertically on ground near the trunk of the tree and height was recorded up to apex leaf of the plant. Girth of the plant (m) - The girth of trees was measured with the help of measuring tape at a height of 12'' above ground level. Plant spread (m) - The canopy/ plant spread of the plant was measured by recording the spread of the plant foliage in East-West and North-South directions. Number of leaves- Number of leaves was counted from each plant of varieties under investigation at the time of initiation of study. Size of pinnae (cm) - Randomly selected five pinnae was taken and length as well as breadth of the pinnae was measured with the help of measuring scale.

Floral characters

The number of ovaries (swelling structure) was counted and subsequently, the numbers of fruits at pea stage (chimiri stage) and at Doka stage were again counted to ascertain fruit set and fruit drop. The fruit set and fruit drop were calculated using the formula;

$$Fruit\ set\ =\ 100\ \times\ \frac{Total\ number\ of\ fruits\ at\ pea\ stage\ (per\ bunch)}{Total\ number\ of\ ovaries}$$

$$Fruit\ set\ =\ 100\ \times\ \frac{Total\ number\ of\ fruits\ at\ harvest\ per\ bunch\ ((doka\ stage}{Total\ number\ of\ fruits\ at\ pea\ stage\ (per\ bunch)}$$

Number of bunches per plant-The fruitful bunches were counted at fruiting stage from each plant in all the cultivars. The average number of bunches per plant was calculated for each cultivar. Length of bunch (cm) - Randomly 4 bunches from each tree were measured for the length of bunch and mean value of each tree was taken into consideration for statistical analysis. Bunch weight (kg) - Three randomly selected bunches were weighed with the help of balance and average weight (kg) of each bunch was worked out. Number of strands per bunch- Number of strands was counted from randomly selected three bunches from which average number of strands per bunch was calculated. Lengths of strand (cm) - Three strands were selected and the length of strand was measured and average length of strand was calculated. Numbers of berries per strand- Three strands were randomly selected from a bunch and number of berries of each strand was counted at the time of harvesting and then average number of fruits per strand was worked out. Weight of berry (g) - Five matured fruits tree - 1 were selected to quantify the weight of berry (g) at doka stage. The fruits were weighed with the help of balance and average weight of single berry was calculated. Stone weight (g) - Stones from the fruits selected for measuring size were separated out and weighed and average stone weight was calculated. Pulp-stone ratio- The pulp- stone ratio was calculated using the following formula;

$$Pulp\text{-}stone\ ratio\ =\ \frac{Pulp\ weight\ (g)}{Stone\ weight\ (g)}$$

Biochemical parameters were assessed viz. T.S.S. through hand refrectometer (0-50° Brix) (AOAC, 1990), titrable acidity using Phenophthalein as an indicator (AOAC, 1990), reducing sugar (Nelson's method), total sugar (Anthron reagent) and ascorbic acid (Colorimetric micro method, Grick et. al., 1953). Boichemical parameters were pooled and workout cluster analysis using NTSYS software.

RESULTS AND DISCUSSION

Growth parameters

Analysis of the morphological parameters revealed that the maximum plant height (4.53 m) was recorded in cv. Dayari whereas, minimum in cv. Khuneizi (3.56m). Cultivar Halawy showed highest girth of plant as compared to other investigated cultivars and the difference among the cultivars were statistically significant. The data presented in Table 1 depicted that maximum plant spread was recorded in cv. Khalas (5.20x4.91m) and minimum in cv. Khuneizi (4.57x4.40m) which were significantly different from each other. Maximum number of leaves plant[-1] (29.75) was observed in cv. Dayari and minimum in cv. Khalas and Khuneizi (19.25 each). The maximum plant girth was observed in Halawy (2.31 m) followed by Sewi (2.21m), Dayari (2.08 m), Khalas (2.06 m) and Khuneizi (1.66 m). The maximum length (43.48 cm) and breadth of pinnae (3.45 cm) were recorded under the variety of Dayari and Halawy respectively (Table-2). The results of present study are in agreement with the finding of Mertia and Vashishtha (1985) while studying the performance of date palm cultivars under desertic conditions of Jaisalmer district of Rajasthan.

Table 1: Growth characters of different date palm cultivars

Cultivars	Height of plant (m)	Girth of plant (m)	Plant spread (m) N-S	E-W
Halawy	4.34	2.31	5.00	5.00
Khalas	4.34	2.06	5.20	4.91
Sewi	3.73	2.21	512	4.88
Khuneizi	3.56	1.66	4.57	4.40
Dayari	4.53	2.08	5.11	4.91
SEm±	0.12	0.11	0.08	0.11
CD at 5%	0.38	0.33	026	0.38

Table 2: Comparison of date palm cultivars on the characteristics of leaves, pinnae, fruit set and fruit drop

Cultivars	Leaves plant [-1]	Length of pinnae (cm)	Breadth of pinnae (cm)	Fruit set (%)	Fruit drop (%)
Halawy	25.75	35.90	3.45	75.84	22.41
Khalas	19.25	37.96	3.04	51.77	47.98
Sewi	24.50	37.55	2.91	72.10	34.91
Khuneizi	19.25	35.42	2.69	79.54	21.49
Dayari	29.75	43.48	2.81	88.50	21.50
SEm±	1.08	1.25	0.11	1.60	1.51
CD at 5%	3.32	3.85	0.33	4.94	4.66

Table 3: Bunch characters of different date palm cultivars

Cultivars	Bunches plant [-1]	Length of bunch (cm)	Bunch weight (kg)
Halawy	6.00	63.50	4.16
Khalas	8.75	57.25	1.93
Sewi	6.25	61.50	1.86
Khuneizi	5.50	67.92	1.90
Dayari	7.75	69.00	2.25
SEm±	0.37	2.08	0.11
CD at 5%	1.15	6.41	0.33

Table 4: Strand parameters of different date palm cultivars

Cultivars	Strands bunch[-1]	Length of strand (cm)	Berry strand[-1]	Weight of berry (g)
Halawy	35.00	36.34	21.34	7.80
Khalas	50.40	31.08	10.06	9.08
Sewi	25.50	43.25	12.67	11.39
Khuneizi	27.08	28.42	15.53	9.17
Dayari	40.17	38.83	10.48	11.30
SEm±	2.14	2.27	0.63	0.42
CD at 5%	6.60	7.00	1.95	1.29

Table 5: Physico-chemical characteristics of different date palm cultivars

Cultivars	Stone weight (g.)	Pulp-stone ratio	TSS°brix	Acidity(%)	Ascorbic acid (mg/100g pulp)	Total sugar(%)
Halawy	1.11	4.18	39.97	0.23	3.73	36.05
Khalas	0.89	7.98	35.30	0.50	3.57	34.17
Sewi	1.13	6.83	32.67	0.30	2.78	33.00
Khuneizi	0.89	9.57	35.03	0.78	2.85	36.25
Dayari	1.83	4.99	32.34	0.58	3.68	31.09
S Em±	0.06	0.33	1.13	0.03	0.39	1.08
CD at 5%	0.18	1.01	2.40	0.07	0.84	2.30

Bunch and fruit characters

Data given in Table 2 revealed that the highest fruit set and length of bunch was recorded in cv. Dayari 88.50% and 69.00 cm, respectively. Whereas, least in cv. Khalas (51.77% and 57.25 cm) in both the parameters. The highest fruit drop was recorded in Khalas (47.98%) and lowest in Khuneizi (21.49%). The maximum number of bunches were recorded in cv. Khalas (8.75) and least in Khuneizi (5.50). The maximum bunch weight was found in cv. Halawy (4.16 kg.) (Table 3) and number of strands per bunch was highest in cv. Khalas (50.40) and least in cv. Sewi (25.50) (Table 4). Similarly the maximum number of berry strand[-1] was observed in Halawy (21.34) and least in Khalas (10.06). Maximum weight of berry was recorded in cv. Sewi (11.39 g) followed by Dayari (11.30 g), Khuneizi (9.17g), Khalas (9.08 g) and Halawy (7.80 g). The highest stone weight was observed in Dayari (1.83g) and it was lowest in Khalas and Khuneizi (0.80g each) while maximum pulp-stone ratio was found in cv. Khuneizi (9.57) and lowest in cv. Khalas (7.98). The resultant plants produce maximum vegetative growth and yield might be due to the genetic makeup of the plant grown the different varieties growing under the same environmental conditions. Similar results were also reported by Mertia and Vashishtha (1985) while studying the performance of date palm cultivars under desertic conditions of Jaisalmer district of Rajasthan.

Biochemical analysis

The highest TSS and ascorbic acid was recorded in cv. Halawy 39.97⁰ Brix and 3.73 mg/100g pulp respectively. Whereas, lowest in cv. Dayari and Sewi (32.34 ⁰B, 32.67⁰B and 3.68,

Table 6: Similarity coefficient for morphological parameters of date palm cultivars

Cultivars	Halawy	Khalas	Sewi	Khuneizi	Dayari
Halawy	0.00				
Khalas	9.21	0.00			
Sewi	4.53	8.81	0.00		
Khuneizi	3.53	1.03	5.17	0.00	
Dayari	4.63	1.07	6.57	5.33	0.00

2.78 mg/100 g pulp, respectively) in both the parameters (Table 5). Differences among the cultivars for the T.S.S. were statistically significant except cv. Sewi and Dayari which were at par. Titratable acidity of doka stage fruits was recorded highest in cv. Khuneizi (0.78%) and lowest in cv. Halawy (0.23%). Sugar content i.e. total, reducing and non-reducing was superior in cv. Khuneizi (36.05%, 32.15% and 3.90%, respectively) followed by cv. Halawy, Khalas, Sewi and Dayari. The similar findings are also reported by Mertia and Vashishtha (1985) in date plam.

Cluster analysis for morphological and biochemical parameters

The morphological data indicated that cv. Halawy is more closely related to cv. Khalas, followed by cv. Dayari and cv. Sewi. Similarly, cv. Sewi shows more close relationship with cv. Dayari and cv. Khuneizi (Table 6). Cultivar Halawy and Khuneizi showed the great affinity and form a cluster A1 with cv. Sewi having close affinity based on similarity coefficient. The cultivars of cluster A1 shown a closer affinity with Dayari and formed a cluster A2. Cultivar Khalas was distantly related to all the investigated cultivars and formed a separate cluster
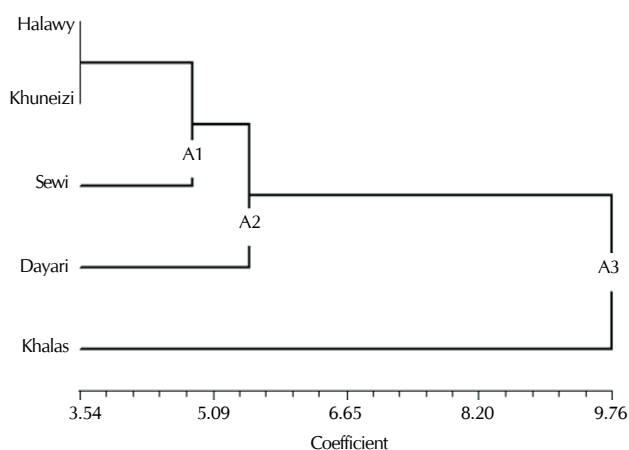
Figure 1: Dendrogram based on morphological parameters

Figure 2: Dendrogram for date palm cultivars using biochemical parameters

A3 (Fig 1). Similar results were also observed in Japanese plum by Mir *et al*. (2009); Bhat *et al*. (2013) in apple and pear and Gothwal (2013) in date palm. Cluster analysis of biochemical parameters represented the results as cv. Sewi and Khuneizi were closest to each other among the investigated cultivars (Fig. 2). The cultivars Dayari and Halawy were found to be closer and made cluster B1 with *cv.* Sewi and Khuneizi. Cultivar Khalas is distantly related to all the investigated cultivars in evolution. Same results are also reported by Srivashtav et al., 2013; Gothwal (2013) in date palm.

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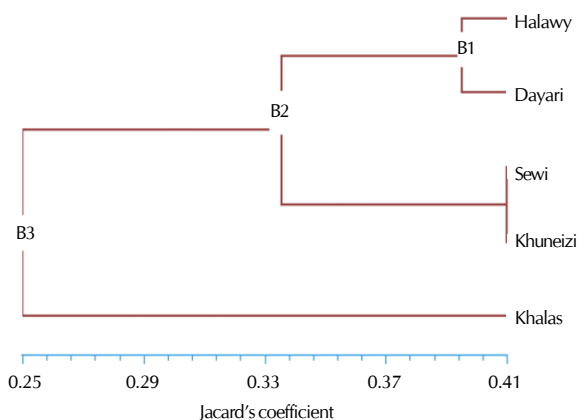
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