



Assessment of male date palm (*Phoenix dactylifera*) genotypes based on morphological and chemotypic attributes

R K MEENA^{1*}, CHET RAM¹, R P MEENA¹, M K BERWAL¹, SHEETAL RAWAT¹,
M K CHOUDHARY¹, D SINGH¹, R S SINGH¹ and A K SINGH²

ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan 334 006, India

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) is a perennial dioecious plant of hot arid ecosystem, commercially cultivated for edible fruits. Non-synchronous flowering phenomenon in date palm is one of the major challenges, therefore, artificial pollination is essentially required for successful fruit setting. Very scanty and scattered information to identify the superior male date palm genotypes as potential pollinisers based on morpho-biochemical characterisation is available. Therefore, the present study was performed to characterise nine male date palm genotypes based on phenotypic and biochemical parameters during 2020–22 at ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan. The carpological and morphological measures of leaf, spathe and inflorescence showed significant variation. The results revealed that CIAH-DP-M8 exhibited the maximum leaf length (400 cm) whereas, the CIAH-DP-M5 exhibited the shortest leaves (250.0 cm). The CIAH-DP-M1 showed superiority in all the recorded parameters related to the inflorescence among the genotype studied. Similarly, in the biochemical parameters also, it was significantly superior in total phenolic content (11.90 mg GAE/g), total antioxidant capacity (16.35 mg AAE/g DW), flavonoids (0.496 mg E.Cat./g DW) and total sugar content (80.90 mg/g) which proved its potential for quality pollen grains production of date palm. The characterised promising male date palm genotype in the present study could serve as potential polliniser in western India.

Keywords: Biochemical potential, Date palm, Morphological diversity, Pollinisers

Date palm (*Phoenix dactylifera* L.), one of the oldest cultivated fruit plant belongs to family Arecaceae. Based on archaeological surveys and other evidences 'Iraq' is considered the centre of origin of date palm (Johnson *et al.* 2013). It has diploid chromosome number ($2n = 2x = 36$) and ~670 Mb genome size (Al-Mssallem *et al.* 2013). The commercial cultivation of date palm has been adopted and acclimatised worldwide in hot-arid regions. The tolerance towards high temperatures, water stress and salinity of the date palm among the fruit crops make it very significant in majority of the desert ecology (Chao and Krueger 2007). Globally, the plantation of date palm covers an area of around 1.23 mha with production and productivity of 8.57 mt and 7.65 t/ha, respectively (FAO 2020). In India, date palm is mainly cultivated in western arid and semi-arid tracks, which is distributed from Kutch region of Gujarat, Rajasthan to some parts of the Punjab and Tamil Nadu (Shah 2014). Due to the non-synchronous flowering

and dioecious nature of date palm, artificial pollination is the prerequisite to obtain superior quality and quantity of fruits (Abu-Zahra and Shatnawi 2019). In north-western part of Rajasthan, male plants generally bear flower during January to March, while female plants bear flowers during February to March. Under such circumstances, natural pollination happens very low, leading to low fruit yield. To overcome fruit setting and crossing, hand pollination is very important and prerequisite in date palm. To fulfil the requirement sufficient numbers of male plants are required for effective pollination (Shah 2014).

The size and shape of the seed as well as size, colour and timing for fruit ripening is directly influenced by the pollen grains used for pollination. The development of date fruits is precisely definite, but varies with the particular male pollinisers used to fertilise the female flowers. This phenomenon has been proven through metaxenia since 1928 by Nixon (1928). Keeping this in view, characterisation and evaluation of pollen grains as superior polliniser for each female cultivar is the need of the hour to establish an intensive programme to produce superior (highly potent) males through different procedures. Availability of viable and quality pollen grains is one of the major constraints

¹ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan; ²Central Horticultural Experiment Station (ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan), Godhra, Gujarat. *Corresponding author email: rkmeena8119@yahoo.com

to harvest economic fruit yield as well as in expansion of date palm cultivation area. To address this issue, attempts were made for identification of elite male pollinisers that is essentially required for further multiplication to meet the quality pollen demand. Moreover, a large set of phenotypic data is required to characterise and evaluate the genetic diversity which is often difficult to assess as sometimes the phenotypic variability is governed by the environmental factors (El Kadri *et al.* 2019). Considering these issues, a comprehensive morphological and biochemical assay was designed with intent to assess the diversity and further conserve the suitable male date palm trees for early flowering and high pollen grains production under hot arid conditions.

MATERIALS AND METHODS

The experiment was conducted during 2020–21 and 2021–22 at ICAR-Central Institute for Arid Horticulture, Bikaner (located at 28°1'N latitude, 73°18'E longitude and at an altitude of 234.84 m above sea level), Rajasthan. Nine promising variants of male date palm, including eight (CIAH-DP-M1, CIAH-DP-M2, CIAH-DP-M3, CIAH-DP-M4, CIAH-DP-M5, CIAH-DPM6, CIAH-DP-M7, CIAH-DP-M8) genotypes and one known variety (Ghanami) were used as planting material. The genotypes were maintained at field gene bank of ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan and further used for other observations. For morphological characterisation, the nine elite male date palms seedlings were evaluated for 34 parameters. Three leaves and three inflorescences of each genotype were considered as standard for morphological description as described earlier by Elhoumaizi *et al.* (2002) and Simozrag *et al.* (2016). Leaves were taken from the mid of crown during pollination. The quantitative traits were measured in all the collected samples of each genotype and average of each trait was calculated. Similarly, the qualitative traits were also recorded for each sample. All the measurements were performed using digital calliper, precision weighing balance and digital measuring tape.

For biochemical analysis, pollen grains from the all nine genotypes were used separately for sample preparation. 200 mg pollen grains were homogenised in 10 mL of 70% methanol, macerated in liquid nitrogen (-196°C) with the help of a mortar and pestle and further incubated at 70°C in water bath for 1 h. Subsequently, tubes were subjected to centrifugation at 10,000 rpm for 10 min at 4°C, supernatant was collected and pellet was discarded for further process. The residue was re-extracted twice with 5 mL of 70% methanol by centrifugation process. The supernatant was pooled and made up the volume up to 20 mL with extraction solvent and stored at -20°C for further use. Total phenol content (TPC) in aqueous methanolic extract of different date palm pollens were estimated following Folin Ciocalteu method as described by Berwal *et al.* (2021). Total flavonoids content (TFC) in aqueous methanolic extract of date palm pollens were estimated by aluminium chloride based colorimetric method (Medini *et al.* 2014), total antioxidant activity (TAA) of date palm

pollens was determined by cupric ion reducing capacity (CUPRAC) assay according to Apak *et al.* (2004). Proline content was measured in aqueous methanolic extract of date palm pollens, following the method described by Carillo and Gibon (2011). Total soluble sugars (TSS) in aqueous methanolic extract of date palm pollens were estimated following the phenol sulphuric acid method (Dubois *et al.* 1956). Reducing sugar concentration in aqueous methanolic extract of date palm pollens was estimated following the method described by Somogyi (1952).

All morphological and biochemical parameters were analysed in a completely randomised design with pollinisers as treatments. The analysis of variance (ANOVA) was conducted using IBM SPSS statistics software v. 20 to test the significance of variation between cultivars for each characteristic.

RESULTS AND DISCUSSION

Morphological characters of date palm genotypes: The male date palm genotypes exhibited significant morphological differences for all characteristics of leaves and male inflorescences studied. The genotype CIAH-DP-M8 registered the maximum leaf length (400.0 cm) whereas, CIAH-DP-M5 registered the shortest leaves (250.0 cm). Genotype CIAH-DP-M1 produced the widest leaf (90 cm) with leaflet length (69.7 cm) and leaflet width (3.7 cm) as compared to other genotypes. The minimum number of thorns were recorded on CIAH-DP-M4 (17.0) whereas, CIAH-DP-M2 produced maximum number of thorns (31.0) (Table 1).

The data (Table 1) also revealed that CIAH-DP-M1 produced the longest thorn (15 cm) and CIAH-DP-M2 (5.0 cm) produced the shortest ones. CIAH-DP-M6 and CIAH-DP-M5 showed maximum inter thorn distance (9.7 cm and 9.4 cm respectively) as compared to all genotypes tested. CIAH-DP-M8 was identified having maximum spine part length (107.0 cm). Four type of thorn attachment were observed in entire the genotypes, viz. single in CIAH-DP-M3; double in CIAH-DP-M1, CIAH-DP-M2, CIAH-DP-M6, CIAH-DP-M8; close to double in CIAH-DP-M5, CIAH-DP-M7 and Ghanami, whereas CIAH-DP-M4 showed mixed type of thorn attachment pattern. The leaf folding angles were found to be wide in CIAH-DP-M2, CIAH-DP-M3, CIAH-DP-M4 and CIAH-DP-M8; medium in CIAH-DP-M5, CIAH-DP-M7 and Ghanami while, CIAH-DP-M1 and CIAH-DP-M6 exhibited narrow leaf folding angle (Table 2). The date of flower emergence started from 1st week of January to the 2nd week of February. Genotype CIAH-DP-M1 was found to be earliest in flowering while CIAH-DP-M3 was last to flower i.e. in 2nd week of February. In CIAH-DP-M2, spathe opening took place during 3rd week of February (earliest) while, in CIAH-DP-M3, it opened during 1st week of March (Table 2).

The examined nine male date palm genotypes revealed a high degree of morphological variation. Numerous previous studies have demonstrated that phenotypic features, especially male genotypes, can be used to differentiate date

Table 1 Morphological parameters of male date palm

Genotypes	Leaf length (cm)	Leaf width (cm)	Leaflet length (cm)	Leaflet width (cm)	Number of thorns	Thorn length (cm)	Inter thorn distance (cm)	Spine part length (cm)	Leaflet area	Number of leaflet/leaf
CIAH-DP-M1	380.0	90.0	69.7	3.7	21.0	15.0	8.7	86.0	294.0	170.0
CIAH-DP-M2	337.0	75.0	46.3	2.9	31.0	5.0	6.0	82.0	255.0	224.0
CIAH-DP-M3	290.0	72.0	46.7	2.8	19.0	6.9	4.9	60.0	230.0	175.0
CIAH-DP-M4	320.0	72.0	42.3	2.4	17.0	10.8	6.0	63.0	257.0	173.0
CIAH-DP-M5	250.0	70.0	39.3	3.2	23.0	7.2	9.4	64.0	186.0	158.0
CIAH-DP-M6	271.0	65.0	46.7	3.1	18.0	9.8	9.7	62.0	209.0	135.0
CIAH-DP-M7	335.0	60.0	46.0	3.8	25.0	8.5	9.0	84.6	250.4	148.0
CIAH-DP-M8	400.0	70.0	46.7	3.6	40.0	8.9	8.8	107.0	293.0	181.0
Ghanami	280.40	68.2	48.4	3.3	22.2	11.2	10.1	63.0	209.2	137.8
SEM ±	4.89	1.78	0.85	0.3	1.57	1.07	1.07	2.26	3.91	1.63
CD ($p=0.05$)	14.15	5.14	2.45	0.10	4.53	3.11	3.09	6.54	11.30	4.70

Table 2 Phenotypic and carpological characteristics of male date palms

Genotypes	Thorn attachment	Leaf folding angles	Date of flower emergence	Date of spathe opening
CIAH-DP-M1	Double	Narrow	1 st week of January	2 nd week of February
CIAH-DP-M2	Double	Wide	3 rd week of January	3 rd week February
CIAH-DP-M3	Single	Wide	2 nd week of	1 st week of March
CIAH-DP-M4	Mixed	Wide	1 st week of February	Last week of February
CIAH-DP-M5	Close double	Medium	3 rd week of January	3 rd week of February
CIAH-DP-M6	Double	Narrow	Last week of January	4 th week of February
CIAH-DP-M7	Close double	Medium	1 st week of February	Last week of February
CIAH-DP-M8	Double	Wide	1 st week of February	Last week of February
Ghanami	Double	Medium	1 st week of February	Last week of February

palm cultivars (Elsafy *et al.* 2015, El Kadri *et al.* 2019, Raza *et al.* 2020). Earlier studies have confirmed that several phenotypic traits like leaf length, leaf width, leaflet length, leaflet width, number of thorns, thorn length, size of spathe (Ahmed *et al.* 2011, Elsafy *et al.* 2015), fronds, number of pinnae and spathe, spine length, length of spiny portion of leaf (Hammadi *et al.* 2009) are reliable for distinguishing and description of date palm accessions.

CIAH-DP-M1 was found to be superior in all evaluated parameters for the inflorescence among all the nine genotype studied. It took maximum number of days for spathe opening (39.8) and produced superior spathe with the maximum length (74.40 cm), width (37.4 cm), weight (0.88 g), weight of inflorescence (0.990 g), weight of spadix (1.86 g), number of spadix/plants (26.8), pollen weight/spathe (27.7 g), number of strands/spathe (156.8), number of flower/strands (104.6), number of strands (15), number of flower inflorescence (15,949), length of central axis (50.6 cm), length of peduncle (54.2 cm), pollen yield/plant (744.86) as compared to all the genotypes studied (Table 3 and 4).

Biochemical characters: Due to metaxenic effect, biochemical parameters affect the fruit quality and quality of fertilised date palm (Shahsavari and Shahhosseini 2022). The total phenolic content in pollen grains of genotypes varied from 7.95–11.90 mg GAE/g. The highest amount of total phenol was obtained in the pollen of CIAH-DP-M1 followed by CIAH-DP-M2 genotypes, whereas the lowest amount was obtained in the pollen of CIAH-DP-M4 genotype which showed a significant difference at the significance level of 5% (Table 4). The highest amount of flavonoid was recovered in the pollen of CIAH-DP-M1 and CIAH-DP-M2, whereas lowest in CIAH-DP-M5. The pollen grains collected from CIAH-DP-M3 and CIAH-DP-M7 genotypes exhibited significantly higher value for TAA, than the others and CIAH-DP-M4 had lowest TAA. The highest proline was recorded in pollens of CIAH-DP-M5 which was significantly higher than the rest of genotypes and the lowest was in CIAH-DP-M2. The maximum TSS content was recorded in CIAH-DP-M2 followed by CIAH-DP-M8, but it was not found to be significantly different from CIAH-DP-M3 and CIAH-DP-M6 genotypes. The lowest

Table 3 Carpological parameters of nine male date palm genotypes

Genotypes	Days taken for spathe opening	Length of spathe (cm)	Width of spathe (cm)	Weight of spathe (g)	Weight inflorescence (g)	Weight of spadix (g)	No. of spadix/plants	Pollen weight/spathe (g)	Number of strands/spathe	Number of flower/strands
CIAH-DP M1	39.8	74.40	37.40	0.88	0.99	1.86	26.8	27.7	156.80	104.6
CIAH-DP M2	30.4	41.20	14.40	0.46	0.63	1.10	21.2	13.0	137.00	53.4
CIAH-DP M3	20.8	34.60	14.60	0.45	0.66	1.11	22.6	19.8	110.00	52.4
CIAH-DP M4	23.6	32.60	17.40	0.43	0.49	0.92	15.0	15.6	105.00	48.6
CIAH-DP M5	20.4	43.80	19.60	0.54	0.71	1.25	19.4	10.6	121.00	58.4
CIAH-DP M6	27.2	34.60	22.20	0.50	0.76	1.26	25.0	21.0	113.60	72.6
CIAH-DP M7	21.2	30.00	15.40	0.26	0.26	0.52	15.0	8.8	147.40	51.6
CIAH-DP M8	22.6	35.40	20.80	0.37	0.63	1.00	18.6	10.8	116.60	44.0
Ghanami	21.8	25.00	13.20	0.25	0.26	0.51	9.6	11.8	105.60	51.80
SEM $\pm$	1.46	2.84	1.93	0.06	0.08	0.13	1.87	1.20	6.45	6.96
CD ($p=0.05$)	4.22	8.21	5.59	0.17	0.24	0.39	5.41	3.47	18.67	20.15

TSS content was recovered in CIAH-DP-M5 genotype, which was significantly different from the other cultivars at a probability level of 5%. The maximum amount of reducing sugars was obtained in pollen of CIAH-DP-M1, whereas the lowest in CIAH-DP-M6, registering a significant difference at the probability level of 5% (Table 4). The findings of the present study showed that the pollen of different genotypes had diverged levels of biochemical compounds. The phenolic components and flavonoids in pollen were responsible for antioxidant properties in pollen of date plant (Al-Shahib and Marshall 2003, Abou-Zeid *et al.* 2019). Pollen from cultivars with higher concentrations of these substances exhibit excellent quality traits for fertility and fertilisation of date tree (Shahsavari and Shahhosseini 2022). The CIAH-DP-M1 exhibited higher amount of total phenolics and flavonoid content comparatively. However, it was lower than estimated total phenolic content in date palm pollens reported by Arafat *et al.* (2013) and Farouk *et al.* (2015). Similarly, the genotype CIAH-DP-M3 and CIAH-DP-M5 showed higher content of total antioxidant capacity and proline, respectively. The proline composition in date palm pollen grains was also reported as limited content in previous studies by Hassan (2011) and Saber (2016). The different genotypes of male date palm of the present study demonstrated significant level of variability in the total and reducing sugar content in their pollen grains which was also found corroborate to the study of Hassan (2011).

Population structure: When performing STRUCTURE 2.0 on the dataset, 10 K values were analysed but only eight have given delta K value, a maximum value of the rate of change in the log probability of data using the method of Evanno *et al.* (2005) was observed at K2, the value of delta K was 2.915054, demonstrating that the germplasm can be clustered into two type of population. The second higher value of delta K (2.004134) was observed at K9. The analysis

revealed two major population including ad-mixtures within populations (Table 5 and Fig. 1). Moreover, the purity and ad-mixtures among the genotypes were also examined by the bar length index value. Number of accessions in population I were 4, out of which 2 (CIAH-DP-M7 and CIAH-DP-M8) were found true to type (pure) genotypes and 2 (CIAH-DP-M5 and Ghanami) were found ad-mixture types. Number of accessions in type two populations were five out of which three (CIAH-DP-M4, CIAH-DP-M2 and CIAH-DP-M1) were with approx. The populations I showed more purity as compare to population II which showed less pure type of population (Fig. 2). The findings of population structure analysis corroborated with the results of phylogenetic analysis.

The employed set of morphological and biochemical characteristics reveal that CIAH-DP-M1 showed superiority in all the studied parameters. The prevailed genetic diversity provided an opportunity to improve fruit quality by using different male genotype as polliniser. For more robust evidence, molecular tools may be incorporated in further genetic diversity related studies. Additionally, more male trees from different regions of the country, even from other countries can be involved, for further development.

The present study revealed considerable morpho-biochemical variability among the nine male date palm genotypes, indicating substantial potential for the selection of superior pollinisers. Significant differences in vegetative, reproductive and biochemical traits reflected the diverse genetic potential of the evaluated genotypes. Among them, CIAH-DP-M1 exhibited superior inflorescence attributes, suggesting greater pollen production potential and vigour for further use in date palm improvement programmes. Additionally, this genotype recorded the highest TPC (11.90 mg GAE/g), TAC (16.35 mg AAE/g DW), flavonoid content (0.496 mg E. Cat./g DW) and total sugar content

Table 4 Flowering and biochemical attributes of nine male date palm genotypes

Genotypes	Number of flower/in florescence	Length of central axis (cm)	Length of peduncle (cm)	Pollen yield/ plants (g)	Phenolics (mg GAE/g)	Flavonoids (mg E.Cat./g DW)	TAA (mg AAE/g DW)	Proline (μ mol/g)	Total soluble sugars (mg/g)	Reducing sugars (mg/g)
CIAH-DP-M1	15,949	50.60	54.20	744.8	11.90	0.496	16.35	0.61	80.90	11.63
CIAH-DP-M2	7,441	25.20	21.00	277.4	10.74	0.482	15.61	0.33	160.50	6.39
CIAH-DP-M3	5,772	16.80	20.80	444.6	8.99	0.432	17.55	0.56	133.05	5.18
CIAH-DP-M4	5,089	29.80	18.60	236.4	7.95	0.476	11.34	0.44	116.46	3.67
CIAH-DP-M5	7,013	29.00	25.60	202.6	10.12	0.322	13.71	1.01	60.99	5.81
CIAH-DP-M6	8,151	15.80	21.20	529.0	9.12	0.330	11.68	0.35	133.56	3.46
CIAH-DP-M7	7,566	25.80	15.60	132.0	8.68	0.414	16.46	0.52	124.06	9.16
CIAH-DP-M8	5,131	20.40	11.80	190.0	8.96	0.358	14.97	0.51	134.00	5.65
Ghanami	5,752	16.60	20.40	145.6	9.25	0.328	11.95	0.45	111.49	13.89
SEM $\pm$	889.88	1.67	0.15	39.09	0.19	0.01	0.2	0.01	2.09	0.24
CD ($p=0.05$)	2,575.12	2.37	0.05	113.1	0.56	0.03	0.6	0.03	6.05	0.71

TAA, Total antioxidant activity.

Table 5 Evano table indicating the number of population

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln''(K)	Delta K
1	6	-1,571.800000	3.694320	-	-	-
2	6	-6,533.366667	1,939.786981	-4,961.566667	5,654.583333	2.915054
3	6	17,149.516667	3,314.964118	-10,616.150000	2,304.650000	0.695226
4	6	25,461.016667	10,516.595408	-8,311.500000	14,035.216667	1.334578
5	6	19,737.300000	13,538.959989	5,723.716667	10,082.616667	0.744711
6	6	24,096.200000	12,266.017041	-4,358.900000	6,789.366667	0.553510
7	6	35,244.466667	25,355.686884	-11,148.266667	24,155.633333	0.952671
8	6	22,237.100000	25,016.165847	13,007.366667	43,475.566667	1.737899
9	6	52,705.300000	27,616.928778	-30,468.200000	55,348.016667	2.004134
10	6	27,825.48333	21,854.975139	24,879.816667	-	-

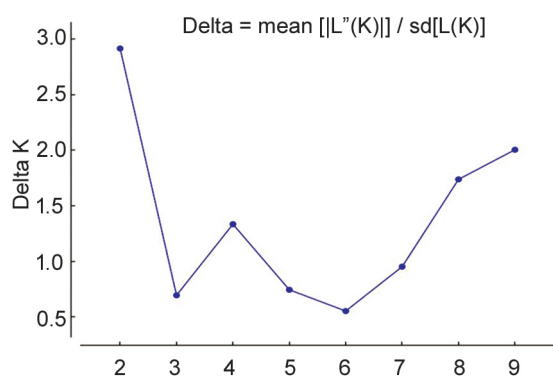


Fig. 1 The analysis showed the ad-mixture of two major populations.

(80.90 mg/g), indicating enhanced biochemical quality. The consistent superiority of CIAH-DP-M1 across both morphological and biochemical parameters highlights its

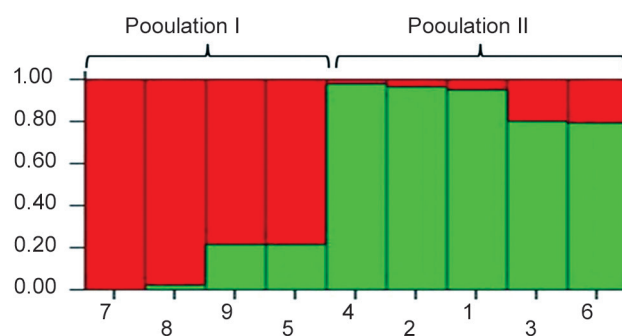


Fig. 2 The graph showing more purity of populations I as compare to population II.

potential as an elite polliniser for breeding and pollination management. Therefore, CIAH-DP-M1 may be considered as a promising polliniser for improving pollination efficiency, fruit set and productivity of date palm under the arid and semi-arid conditions of western India.

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