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Assessment of phenotypic diversity of some date palm male genotypes growing under Egyptian conditions

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Abstract: Artificial pollination is a prerequisite to achieve a sufficient yield of palm trees; hence the identification of superior male palm trees as a standard pollen source for date palm growers has a high priority. The current study was carried out to assess phenotypic diversity among four date palm males namely Hayani, Meghal, Fardh, and Ghannami Ahmer. The genetic diversity and phylogenetic relationships between palm genotypes were performed using eighteen quantitative morphological parameters of both vegetative and reproductive parts (leaf, leaflets, spines, and spathe). Principal components analysis and hierarchical clustering was performed to identify the differences and similarities between the studied genotypes. Results showed significant differences in morphological parameters between the four male palm trees. Most of the selected morphological parameters could be used as quantitative markers for the identification of date palm male genotypes. Principal component analysis showed that, the studied parameters formed two major homogeneous groups of variables, which possessed a high proportion of the observed phenotypic diversity among the studied date palm males. Cluster analysis showed that Fardh, Ghannami Ahmer, and Meghal, are closer related than Hayani; Ghannami and Fardh males showed the highest genetic similarity while the lowest similarity index was detected between Hayani and Ghannami Ahmer males.

Keywords: *Phoenix dactylifera*; pollinizers; phenotypic diversity; morphological identification.

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Avaliação da diversidade fenotípica de alguns genótipos masculinos de tamareiras cultivadas sob condições egípcias

Resumo: A polinização artificial é um pré-requisito para obter bom rendimento de tamareiras; portanto, a identificação de tamareiras masculinas superiores como fonte padrão de pólen para produtores de tamareiras é de suma importância. O presente estudo foi realizado para avaliar a diversidade fenotípica entre quatro genótipos masculinos de tamareiras, a saber: Hayani, Meghal, Fardh e Ghannami Ahmer. A diversidade genética e as relações filogenéticas entre os genótipos de tamareiras foram realizadas usando dezoito parâmetros morfológicos quantitativos das partes vegetativa e reprodutiva (folha, folíolos, espinhos e espata). A análise de componentes principais e do agrupamento hierárquico foi realizada para identificar as diferenças e as semelhanças entre os genótipos estudados. Os resultados mostraram diferenças significativas dos parâmetros morfológicos entre os quatro genótipos masculinos. A maioria dos parâmetros morfológicos selecionados poderia ser usada como marcadores quantitativos para a identificação de genótipos masculinos de tamareira. A análise de componentes principais mostrou que os parâmetros estudados formaram dois grandes grupos homogêneos de variáveis, que possuíam alta proporção da diversidade fenotípica observada entre os genótipos masculinos de tamareira estudados. A análise de agrupamento mostrou que os genótipos Fardh, Ghannami Ahmer e Meghal têm relação mais próxima do que Hayani; Ghannami e Fardh apresentaram a maior similaridade genética, enquanto o menor índice de similaridade foi detectado entre Hayani e Ghannami Ahmer.

Termos de indexação: *Phoenix dactylifera*; polinizadores; diversidade fenotípica; identificação morfológica.

Introduction

Date palm (*Phoenix dactylifera* L., family Palmaceae) is a tree crop of economic, historical and social importance; it is widely cultivated in arid and semi-arid regions, about 100 million date palm trees are growing wide world; 60% of date palm trees are grown in the middle east and north Africa countries (CHAO; KRUEGER, 2007; WAKIL et al., 2015). Egypt is the leading date palm producer in the world followed by Iran and Saudi Arabia (BEKHEET; EL-SHARABASY, 2015). Date palm is a dioecious, cross pollinated species, hence artificial pollination is a critical prerequisite process to ensure enough fertilization and achieve sufficient crop yield; it has been documented that, source of pollen grains has a direct effect on fruit set, yield, and fruit quality (AWAD, 2010; FARAG et al., 2012). Therefore, it is important to identify and select a superior male palm tree as a standard pollen source for date palm growers (RAZA et al., 2020; RIZK et al., 2006). Most of the available pollinating date palm males originate from seed propagation, resulting in many different local males that vary greatly in blooming date, pollen viability and compatibility with the pollinated female trees (JASKANI et al., 2016; RIZK et al., 2006). There is limited information regarding the identification and selection of the male trees of date palm (JASKANI et al., 2015). Most of the female date palm cultivars are recognized by fruit characteristics (fruit weight, size, shape and color *etc*.). However, the identification of male genotypes is a more difficult process because most of the male palm trees are seed propagated and usually named according to the geographic origin or farmers experience (SIMOZRAG

et al., 2016). Assessment of genetic diversity of date palm genotypes would be of major importance in germplasm characterization and conservation (AL-KHALIFAH; SKARI, 2003). Assessment of the genetic diversity among date palm genotypes is performed by morphological parameters (AHMED et al., 2011; NAQVI et al., 2015; SALEM et al., 2008), biochemical (GOMEZ-VIDAL et al., 2008; KHOSHROO et al., 2011;SALEM et al., 2001) or molecular markers (ABDULLA; GAMAL, 2010; AHMED; AL-QARADAWI, 2009; ELSHIBLI; KORPELAINEN, 2008; ZEHDI et al., 2004). Several morphological markers have been used to study the genetic diversity of date palms and discriminate between closely related genotypes (ARABNEZHAD et al., 2012; EL-ASSAR et al., 2005; RACCHI et al., 2013). Morphological markers provided a signature to insure genotype purity of different fruit crops, *i.e.* mango, olive, banana and citrus (DOMINGUES, 1999; GIBERT et al., 2009; ELKHESHIN et al., 2016; BOUCHEFFA et al., 2019). A variety of morphological parameters have been employed for genotypes identification of date palms; leaves, spin, spathes and fruit characteristics are usually used for the identification of date palm genotypes (AHMED et al., 2011; CHAO; KRUEGER, 2007; SALEM et al., 2008). The current study was carried out to assess the phenotypic diversity of some male palm trees (Hayani, Meghal, Fardh, and Ghannami Ahmer) using the quantitative parameters of IPGRI descriptors to identify discriminant descriptors that can be used to differentiate between the studied male palm trees and find out the genetic relationship among them.

Materials and methods

Plant materials and sample collection

The current study was conducted on four male palm genotypes (Hayani, Meghal, Fardh, and Ghannami Ahmer). Hayani and Meghal trees were originated as a seedling trees and named locally according to their vegetative traits by the farmers (EI-GHAYATY et al., 2014; OMAR ; EL-ABD, 2014; SALOMÓN-TORRES et al., 2021) while Ghannami and Fardh Ahmer were imported from Iraq (AL-JIBOURI ; ADHAM,1990) and Sultanate of *Oman* (AL-YAHYAI; KHAN, 2015) respectively. All trees of the studied genotypes were propagated by offshoots; Hayani was grown in a date palm orchard at Bilqas, Dakahlia Governorate, Egypt (31°12’42.8”N 31°21’24.0”E), while the other genotypes were grown in a date palm orchard at Cairo, Alex desert road, Giza Governorate, Egypt (29°53’10.4”N 31°03’47.7”E). The morphological parameters measurements and samples analysis were carried out in the Laboratories of the Horticulture Research Institute, Agriculture Research Center, Egypt.

Morphological characteristics

Eighteen morphological variables (Table 1) were analyzed according to the international descriptors of date palm (IPGRI, 2005) to describe quantitatively the vegetative and reproductive organs; all the measurements were repeated in two successive years; principal component analysis and cluster analysis were performed using the average of the two years. Three healthy uniform date palm trees were selected from each genotype; four years old leaves were collected from different main directions during April; leaf length (cm) was measured from the end of the spine zone to the top of the leaf, the number of leaflets per leaf was counted, the length and width of leaflets (cm) were determined as an average of 10 leaflets per replicate taken from the middle portion of the leaf. Five spathes per palm were collected to measure spathe length and width (cm) at bursting. The length of stalk zone (cm), the number of stalks per spathe, stalk length (cm), the number of flowers per stalk, and pollen weight per spathe (g) were determined. Pollen viability percentage was determined according to Al-Najm et al. (2021) by staining in aceto-carmine (2% w/v carmine dissolved in 45% acetic acid).

Table 1 - Measured vegetative and reproductive parameters in date palm trees

Code	Vegetative parameters	Unit
LV1	Leaf length	cm
LV2	Leaf base width	cm
LV3	Leaf base thickness	cm
LV4	Number of leaflets per leaf	number
LV5	Leaflets length	cm
LV6	Leaflets width	cm
LV7	Length of spine zone	cm
LV8	Number of spines per leaf	number
LV9	Spines length	cm
LV10	Spines thickness	cm

Code	Reproductive parameters	Unit
RV1	Spathe length	cm
RV2	Spathe width	cm
RV3	Length of stalks zone	cm
RV4	Number of stalks per spathe	number
RV5	Stalk length	cm
RV6	Number of flowers per stalks	number
RV7	Pollen weight per spathe	g
RV8	Pollen viability	%

Statistical analysis

Data were statistically subject to analysis of variance (ANOVA), differences among the means, were determined using and least significant differences (LSD) at $p \leq 0.05$ (SNEDECOR; COCHRAN, 1967) using Statistix (8.0) software. Principal component analysis (PCA) was performed to identify parameters with greater contributions to total variability (JOLLIFFE, 2002), hierarchical cluster analysis (SARAÇLI et al., 2013) was performed and displayed in a *heatmap*; PCA and cluster analysis was carried out using XLSTAT (Addinsoft, version 23.5.1227).

Results and discussion

Morphological characteristics

Results of data analysis revealed significant differences between the male genotypes in most of the vegetative characteristics (Figure 1). In this regard, Hayani and Meghal males showed superiority over other male palm trees in most traits. These differences between males may be due to genotypic variability. Hayani palm male tree had the highest significant values of leaf length (518 and 515 cm), leaf base width (27.06 and 26.6 cm), the number of leaflets per leaf (213.3 and 213), and length of spine zone (108.3 and 109 cm) in both seasons, respectively as compared with the other palm tree males. However, Hayani males recorded the lowest leaflet length values (29.8 and 29.6 cm) in both seasons respectively. Meghal palm tree male gave the highest values of leaf base thickness (7.16 and 7.66 cm), leaflet width (3.93 and 3.71 cm), number of spine per leaf (33 and 32.3), and spine thickness (0.92 and 0.93 cm) in both seasons, respectively. On the other hand, Fardh palm tree male recorded the lowest values of number of leaflets per leaf (79.3 and 80), leaflets width (2.17 and 2.16 cm), length of spine zone (72.6 and 74.3 cm) respectively during the two seasons. In comparison, Ghannami palm tree male recorded the lowest values of leaf length (364 and 358 cm) in both seasons, respectively. Data of the spathe, inflorescence, and pollen grain viability are illustrated in (Figure 2). Regarding the differences between the male palm trees, data showed that Hayani palm male trees produced the longest spathe (98.3 and 98.6 cm in both seasons, respectively) than those of the other palm male trees. Also, data clearly showed that Hayani palm male trees had significantly the highest values in length of stalk zone (76.0 and 73.3 cm), stalk length (23.96 and 26.13 cm), and the number of flowers per stalk (74.16 and 65.06) in both seasons, respectively, as compared with the other male trees in the two seasons. Meanwhile, Fardh male gave the lowest significant values for spathe length (49.00 and 51.73 cm), length of stalks zone (44.6 and 46.6 cm), and the number of flowers per stalk (50.96 and 51.73) in both seasons respectively. In addition, Ghannami palm tree male recorded the highest values of pollen grains weight per spathe (49 and 48g in both seasons, respectively). Meanwhile, the other palm male trees had intermediate values of pollen grains weight with no significant difference between Hayani and Fardh palm

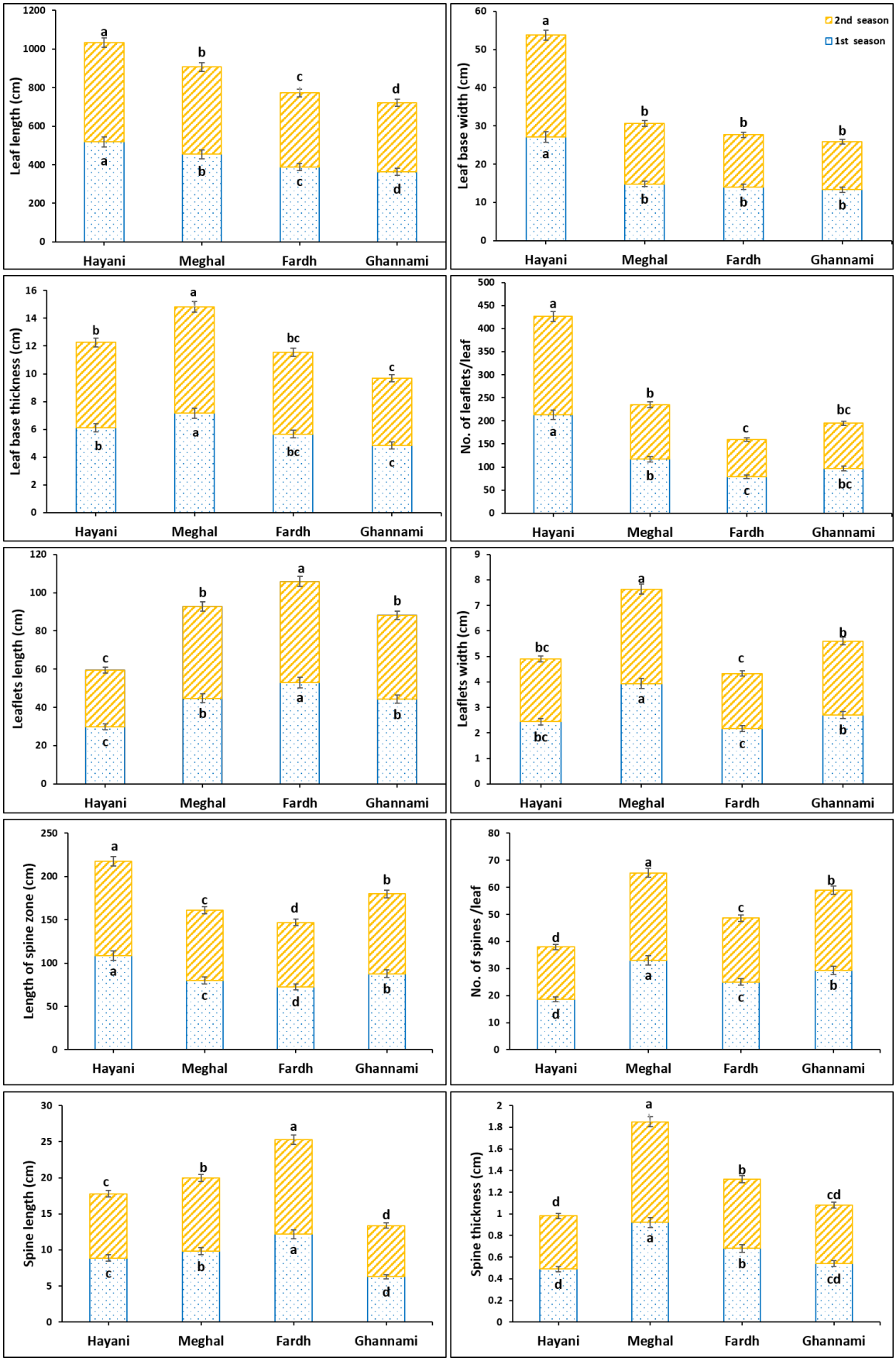

Figure 1- Leaf parameters of Hayani, Meghal, Fardh, and Ghannami Ahmer male palm trees in two seasons; all results are expressed as means ± deviation (represented by vertical bars). Means with the same alphabets letters are not significantly different at $P \leq 0.05$.

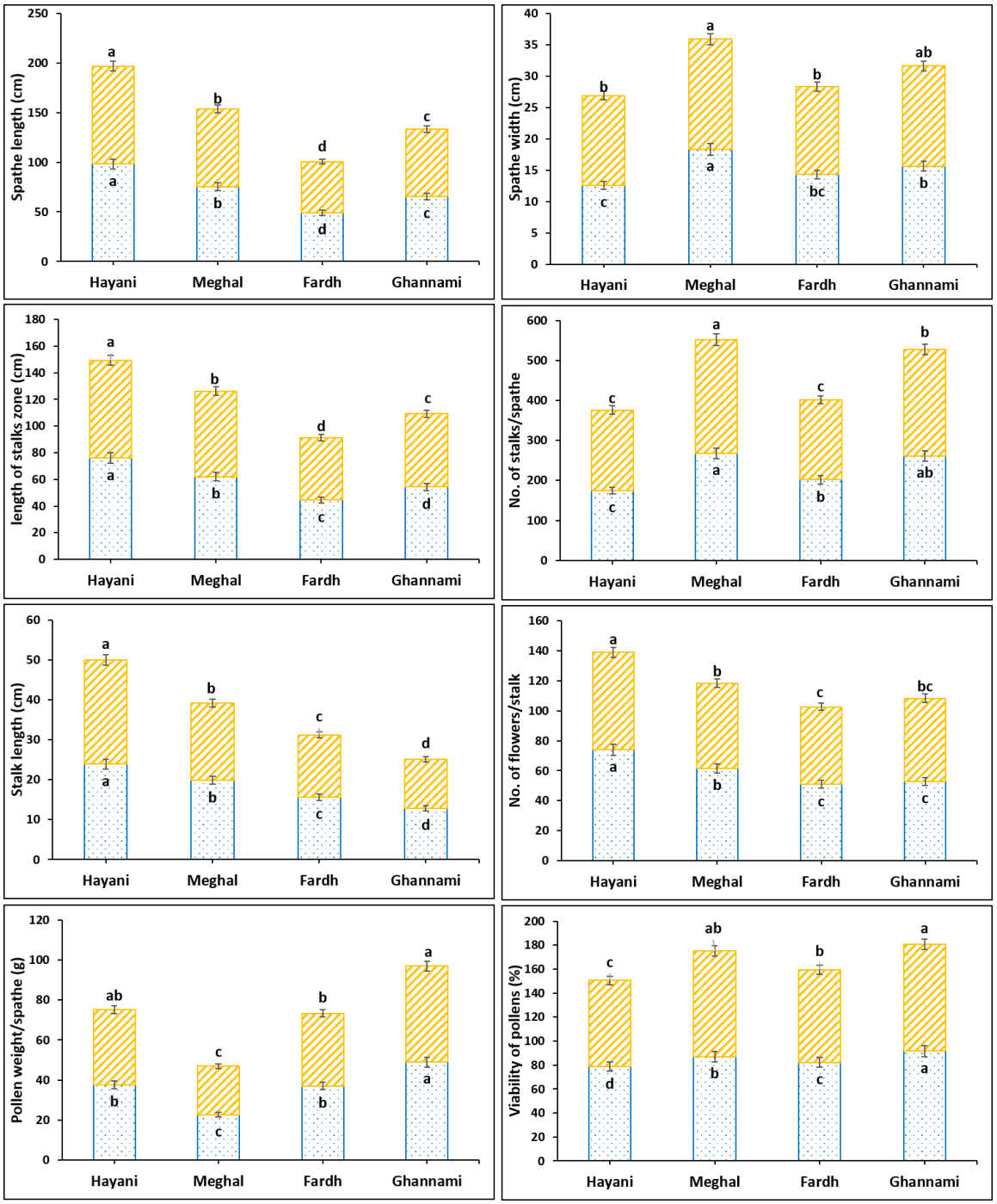

Figure 2 - Spathe characteristics of Hayani, Meghal, Fardh, and Ghannami Ahmer date palm male trees in two seasons; all results are expressed as means ± deviation (represented by vertical bars). Means with the same alphabets letters are not significantly different at $P \leq 0.05$.

male trees. However, pollen viability using the aceto-carmen staining test revealed that Ghannami Ahmer palm male tree had significantly the highest value of pollen viability in both seasons (91.7 and 89.1 %, respectively). Several vegetative parameters, including leaf length, number and length of leaflets, number of spathes, spine length, and length of spiny zone are reliable parameters for differentiation and description of date palm accessions (EISSA et al., 2009; HAIDER et al., 2015; HAMMADI et al., 2009; SAKR et al., 2010; SALEM et al., 2008). According to Elhoumaizi et al. (2002), vegetative growth parameters depict a high degree of phenotypic polymorphism in date palm trees. The obtained results in the current study revealed that a broad range of variability

was observed in the measured parameters, which enables identification and differentiation between the different date palm genotypes. According to Djerouni et al. (2015), date palm leaf and spathe traits can be used to determine the morphological variability in male date palm accessions.

Principal component analysis

Principal component analysis (PCA) was performed to define the important parameters responsible for the observed variability between the studied date palm genotypes. The principal component of the first and second principal components (PC1 and PC2) accounted for 55.75 % and 28.35% of the total variation respectively (Figure 3). Regarding the contribution of the most important variables, based on PC1 leaf length, leaf base width, number of leaflets per leaf, spathe length, length of stalks zone, stalk length and number of flowers per stalk were positive loading variables, whereas leaflets length and spine thickness had negative loadings. Moreover, PC2 had two distinguished groups of variables; the positive loading variables included leaf base thickness, leaflets width and spathe width, while pollen grains weight per spathe had negative loading. Figure (3) showed a significant diversity between Hayani and the other three genotypes in the first principal component. The graphic representation of variables showed that the studied parameters formed two major homogeneous groups of variables, the first one including leaf length, leaf base width, number of leaflets per leaf, spathe length, stalk zone length, stalk length and number of flowers per spathe. Moreover, the number of spines per leaf and spine thickness were correlated to spathe width, number of stalks and pollen grain viability. Principal component analysis was previously used to study phenotypic diversity and define traits with a major contribution to the observed variability (AHMED et al., 2011; ELHOUMAIZI et al., 2002; HAIDER et al., 2015). The obtained results showed that most of the measured parameters, including both leaf and spine characteristics possessed a high proportion of the observed phenotypic diversity among the studied date palm males.

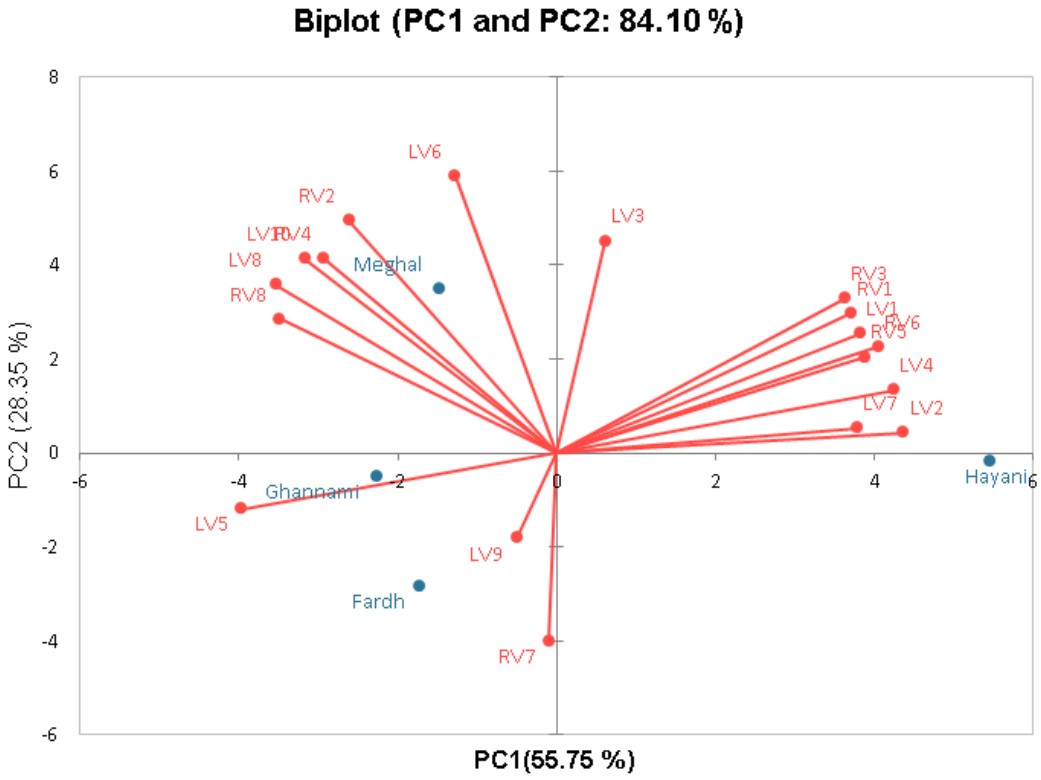

Figure 3 - Principal component analyses (PCA) biplot based on leaf and spathe characteristics of the four date palm males; parameters abbreviations as in Table 1.

Clustering heatmap based on morphological characteristics

Cluster analysis of the relationship between the different parameters and genotypes is displayed as a heatmap (Figure 4). Based on the variations in leaf measurements, the date palm males were grouped into three column-clusters (Figure 4); Fardh and Ghannami Ahmer showed higher similarity index, while Hayani had the lowest similarity with the three other date palm males; leaf parameters were grouped into three row-clusters, the 1st row cluster including leaf length, number of leaflets and length of spine zone; positive correlations were also found between leaf base width, leaflet length and number of spines per leaf which, were assembled in the 2nd row cluster; the 3rd row cluster including spine thickness, leaflet width and leaf base thickness (Figure 4). Analysis of the relationship between different date palm genotypes based on the spathe parameters was slightly different; the date palm males were grouped into only two column-clusters (Figure 4). Spathe length and spathe width were highly correlated parameters and were grouped in the 1st row cluster; positive correlations were also found between pollen weight per spathe, pollen grain viability, spathe length, length of stalk zone and the number of flowers per stalk, which were assembled in the 2nd cluster; the number of stalks per spathe showed a weak correlations with the other spathe parameters (Figure 4). A dendrogram was constructed using the eighteen morphological traits; genetic linkage between the examined date palm males is illustrated in a dendrogram with three main clusters (Figure 5). Fardh, Ghannami Ahmer, and Meghal are closer relatives than the Hayani. The first main cluster contained only Hayani cultivar which was entirely different from all other male palm trees. The second main cluster had two sub-clusters, the first sub-cluster contained Meghal, while the second one contained Fardh and Ghannami Ahmer. Multivariate compound similarity analysis presented as a heatmap is usually utilized to show more information about the genetic variance of plant genotypes (METSALU; VILO, 2015). In the present study, morphological diversity within the male accessions was very high for some qualitative characteristics; the obtained results revealed that some of the measured parameters were not correlated and others had weak correlations, while some parameters were highly correlated. According to

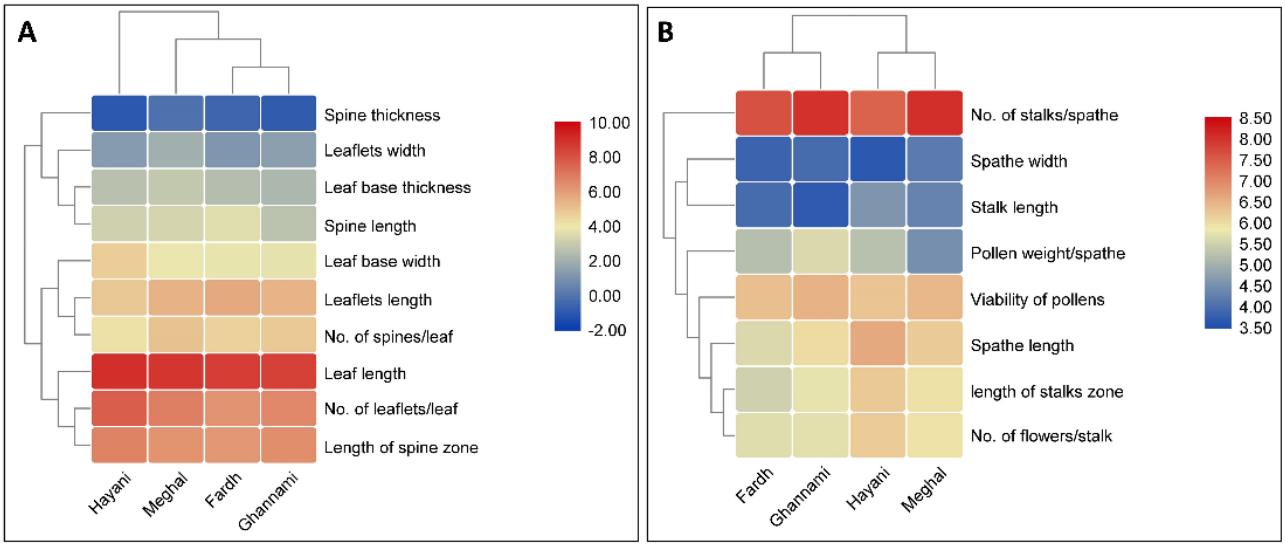

Figure 4 - Hierarchical clustering with heatmap illustrating the relationships between Leaf (A) and Spathe (B) characteristics of Hayani, Meghal, Fardh, and Ghannami Ahmer palm males; each column represents a genotype, whereas each row represents a variable, red color represents higher relative value and blue color represents lower relative value.

Haider et al. (2015) positive and negative correlations exist among morphological traits of date palm trees. Heatmap analysis revealed that leaf measurements exhibited greater variation among the studied date palm and had a higher potential for discriminants between date palm genotypes compared with the spathe measurements, which was confirmed by the results of the hierarchical clustering using all morphological characteristics (Figure 5). The results of variation in morphological characteristics of the date palm male genotypes were previously reported (RABIE, 2007; IBRAHIM et al., 2013). The variation regarding the morphological characteristics of studied male genotypes reflects the differences in their genetic constituents. Also, the correlation coefficient can provide information on the traits that are most important in assessing accession diversity (MILATOVIĆ et al., 2010 ; NORMAN et al, 2011).Similar studies on the morphological diversity of date palm (AHMED et al., 2011; HAMMADI et al., 2009; SALEM et al., 2008), fig (SADDOUD et al., 2008) and olives accessions (QURESHI et al., 2020) had been reported previously. Some of the morphological parameters showed a weak correlation *e.g.*, leaf base thickness has a weak correlation with leaflets number, leaflets length, spines number, and spine thickness. Likewise, leaflet width has a weak correlation with the length of the spine zone and spines length. Similar results were recorded for the spaths measurements; spathe length has a weak correlation with the number of stalks per spathe, also, pollen grain weight per spathe has a weak correlation with both of the number of flowers per stalk and the pollen grains viability. Previous studies revealed that most of the measured vegetative parameters in date palm trees have weak correlations (BEDJAOUI; BENBOUZA, 2020; HAIDER et al., 2015). Similar results were reported for olive (BOUCHEFFA et al., 2019), walnut (ARZANI et al., 2008) and cherry (PETRUCCELLI et al., 2013).

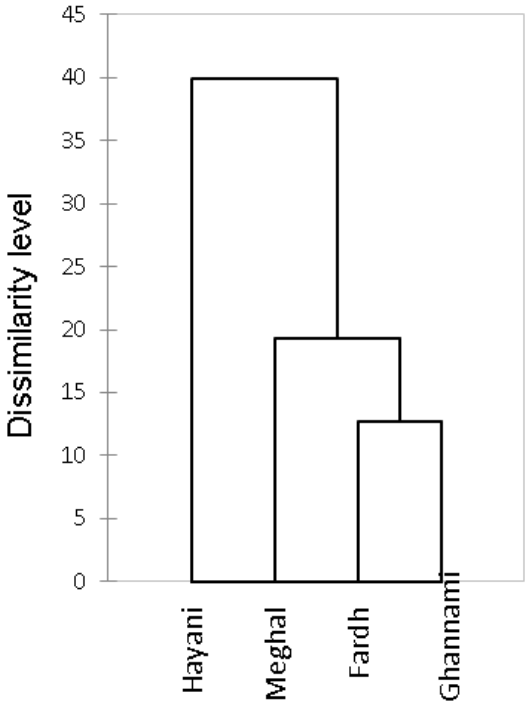

Figure 5 - Dendrogram of hierarchical clustering of four palm male trees based on morphological characteristics.

Conclusion

We can conclude that a broad range of variability was observed in the measured vegetative parameters, which enables identification and differentiation between the date palm genotypes. The principal component analysis showed that the studied parameters formed two major homogeneous groups of variables; most of the measured parameters, including both leaf and spine characteristics possessed a high proportion of the observed phenotypic diversity among the studied date palm males. Genetic linkage between the examined date palm males is illustrated in a dendrogram with three main clusters. Fardh, Ghannami, and Meghal are closer related than the Hayani, which is entirely different from all other male palm trees.

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