Analysis of the Morphological Diversity of Inflorescence of Six Tunisian Date Palm (Phoenix dactylifera L.) Pollinators

K. Kadri, K. Aounallah, and M. Abdelhafidh

ABSTRACT

Six Tunisian date palm pollinators were morphologically characterized at the inflorescence level. The aim was to assess the level of similarity between the spathe of the three flowering stages. Nineteen qualitative and quantitative characteristics were explored and subjected to multivariate analyzes. The results showed that inflorescences morphological similarity oscillated between 97.9 and 62.2% with an average of 80.1%, which testifies strong genetic relations among the six genotypes and among flowering stages/cycles within each genotype. Principal Component Analysis (PCA) analysis shows that weight of the spathe (SW), number of spathe (NS), shape of spathe (SS), total length of the spathe (TLS), shape of spikelets (SPS), number of spikelets per spathe (NS), These characteristics are defined by axis 1 and 2 which absorbing a total of 56.3% of total variability. Phylogenetic relationship analysis showed that two groups were distinguished by a high level of genetic similarity of inflorescences at each flowering stage. According to the analysis, the second flowering stage is the most distinguished morphologically with the highest sizes of the quantitative characteristics studied.

Keywords: Date palm, Dendrogram, Flowering, Inflorescences, Quantitative and qualitative traits, Pollinator, PCA.

I. INTRODUCTION

The cultivation of the date palm (Phoenix dactylifera L.) occupies an important place in the agricultural production system in Tunisia and ranks second in terms of exports of agricultural products after olive oil [1]. This crop representing a major source of income for farmers and related industries in several oasis located in the South West of Tunisia [2]. As a dioecious specimen, date palm is at the origin of an exceptional varietal richness [3] where each seed sown can indeed constitute a new variety that makes counting the existing varieties a more difficult process [4]. The Tunisian phoenicultural heritage is characterized by a great varietal diversity represented by more than 300 varieties [5]. The traditional oasis ecosystem constitutes a reservoir for the genetic diversity of the date palm crop which is now threatened with disappearance for various causes (siling up, lack of water, aging, monovarietal phoenici culture, etc.). Since antiquity, the selection and the distinction only affect the female date palm, in Tunisia; it was rarely question of selection and characterization of the male date palm, locally named “Dokkars”. In this context, a certain number of works have been carried out on the characterization and evaluation of some populations of dokkars, we cite: the work of Karim et al. [6]; Kadri et al. [7]; Kadri et al. [8]; Eldakri et al. [9]. In Tunisia, the flowering of male spathe begins from the end of February until April for most cultivars [10]. Generally, date palm male trees produce their spathes (inflorescences) in cycles/waves during the flowering period that mostly extend to more than a month. However, there is little available information on spathe morphology for each flowering stage. Our work aims to identify the “Dokkars” by morphological characterization of the inflorescences and to assess the genetic diversity existing between the three flowering stages.
II. MATERIALS AND METHODS

A. Plant Materials

Six date palm pollinators were chosen for this study, these cultivars belong to the experimental plot of the regional agricultural research center of Degache (E: 33°58′40.5″; N: 008°12′31.8″). The pollinators have the same age category and undergo the same cultivation technique (fertilization and irrigation ...). Each male foot is presented by a code: ABD1, P4, P13, P3, P7 and P8 (Fig. 1).

B. Morphological Characterization of Inflorescence

The morphological characterization of the inflorescences of the six male date palm genotypes (Fig. 2) at each flowering stages/cycles (early, medium, and late) during the flowering period were performed according to the parameters listed in the descriptor of the date palm of IPGRI [11] (Table I), using inflorescences at each flowering cycle for each genotype. These phenotypic characteristics of the inflorescences were used to establish a descriptive sheet of the various male genotypes, the collection of male spathes was done just one day after flowering, for each stage three inflorescences are collected to carry out the measurements.

C. Statistical Analysis

For the analysis of the morphological diversity of the inflorescences of six pollinators, three repetitions were performed for each flowering stage (early, medium, and late). Principal Component Analysis (PCA) in this study was performed using Multi-Variate Statistical Package software (MVSP 3.22). The coefficient of similarity was used by the NTSYS software to establish the dendrogram corresponding to the grouping of the different six pollinators according to the WPGMA “Weighted Pair-Group Method using Arithmetic” analysis [12].

III. RESULTS AND DISCUSSIONS

A. Principal Component Analysis

Morphological parameters of the male inflorescences (Table II and III) were processed by principal component analysis (PCA) using MVSP software (3022) (Fig.3). Axis 1 (F1) and axis 2 (F2) were chosen, which absorbs the maximum of variability existing among the six male date palm genotypes. The first axis represents 33.4% while the second represents 22.9% of variability which absorbing a total of 56.3% variability. Axis 1 is defined by the following parameters: the weight of the spathe (SW), the density of the spikelets (SD), the shape of the spikelets (SPS), the length of the shortest spikelet (SSL), number of flowers per shortest spikelet (NFLS), the length of the part of the spikelet without fruit in the middle of the spathes (LSFMS) and length of the part of the spikelet with flowers at the top of the spathe (LSFBS). The analysis of the PCA (Fig. 3) shows the existence of distinct groups.

Group A composed of the genotype ‘P3’ at all flowering stages which correlate negatively with axis 2. In fact,
spathe of 'P3' passing from one stage to another and did not significantly stand out from each other and form an agglomeration by the characters which reflect this axis and were distinguished from other genotypes by the same criteria. Group B contained 'P7' genotype at all flowering stages and was positively correlated with axis 1. However, the third group C, included 'P4' genotype at the different flowering stages and was highly correlated with axis 2. Group D correlates positively with axis 2 and negatively with axis 1 and it consists of 'P13' from the various flowering stages.

| Pollinators Descriptors | ABD1 | P4 | P7 | P8 | P3 | P13 |
|-------------------------|------|----|----|----|----|-----|
| Flowering date          |      |    |    |    |    |     |
| 28/02/20                |      |    |    |    |    |     |
| 5/03/20                 |      |    |    |    |    |     |
| 4/03/20                 |      |    |    |    |    |     |
| 29/02/20                |      |    |    |    |    |     |
| 19/03/20                |      |    |    |    |    |     |
| 16/03/20                |      |    |    |    |    |     |
| LSD value               |      |    |    |    |    |     |
| Inflorescences emitted during the first flowering stage |     |
| NS                      | 11\textsuperscript{a}  | 12\textsuperscript{a}  | 10\textsuperscript{a}  | 11\textsuperscript{b}  | 8\textsuperscript{a}  | 12\textsuperscript{a}  | <0.000  | 2.9949337 |
| TLS (cm)                | 137\textsuperscript{b}  | 144\textsuperscript{b}  | 138\textsuperscript{b}  | 100\textsuperscript{a}  | 106\textsuperscript{b}  | 131\textsuperscript{b}  | <0.000  | 3.04534392 |
| TSW (cm)                | 13.5\textsuperscript{a}  | 15\textsuperscript{a}  | 18.5\textsuperscript{a}  | 18\textsuperscript{a}  | 14\textsuperscript{a}  | 14.5\textsuperscript{a}  | <0.000  | 0.95070275 |
| SW (g)                  | 2250\textsuperscript{a}  | 3420\textsuperscript{a}  | 3323\textsuperscript{a}  | 2385\textsuperscript{a}  | 2150\textsuperscript{a}  | 1873\textsuperscript{a}  | <0.000  | 19.2365477 |
| NSS                    | 297.3\textsuperscript{a}  | 258.0\textsuperscript{a}  | 405.3\textsuperscript{a}  | 233.3\textsuperscript{a}  | 302.5\textsuperscript{a}  | 170.6\textsuperscript{a}  | <0.000  | 4.69929082 |
| LLS (cm)                | 41.49\textsuperscript{a}  | 34.49\textsuperscript{a}  | 34.50\textsuperscript{a}  | 37.56\textsuperscript{a}  | 36.76\textsuperscript{a}  | 27.86\textsuperscript{a}  | <0.000  | 0.84154943 |
| NFLS                    | 111\textsuperscript{a}  | 84\textsuperscript{a}  | 77\textsuperscript{a}  | 93\textsuperscript{a}  | 95\textsuperscript{a}  | 72\textsuperscript{a}  | <0.000  | 2.34914541 |
| SSL (cm)                | 7\textsuperscript{a}  | 4.03\textsuperscript{a}  | 8.5\textsuperscript{a}  | 8.33\textsuperscript{a}  | 3.69\textsuperscript{a}  | 5.35\textsuperscript{a}  | <0.000  | 0.58597983 |
| NFSS                     | 14\textsuperscript{a}  | 10\textsuperscript{a}  | 25\textsuperscript{b}  | 21\textsuperscript{a}  | 6\textsuperscript{a}  | 22\textsuperscript{a}  | <0.000  | 2.34914541 |
| LSWFTS (cm)             | 8\textsuperscript{a}  | 3.66\textsuperscript{a}  | 7\textsuperscript{b}  | 5.86\textsuperscript{b}  | 5.33\textsuperscript{b}  | 6.40\textsuperscript{b}  | <0.000  | 1.45128269 |
| LSWFTS (cm)             | 4.43\textsuperscript{a}  | 3.90\textsuperscript{a}  | 6.30\textsuperscript{a}  | 4.90\textsuperscript{a}  | 3.03\textsuperscript{a}  | 2.96\textsuperscript{a}  | <0.000  | 0.3714325 |
| LSWFTS (cm)             | 3\textsuperscript{e}  | 1.16\textsuperscript{a}  | 2.5\textsuperscript{b}  | 2.59\textsuperscript{b}  | 1\textsuperscript{a}  | 2\textsuperscript{a}  | <0.000  | 0.25970779 |
| LSFBS (cm)              | 13.89\textsuperscript{d}  | 12.89\textsuperscript{b}  | 14.30\textsuperscript{b}  | 15.86\textsuperscript{b}  | 11.86\textsuperscript{b}  | 19.33\textsuperscript{b}  | <0.000  | 0.34578484 |
| LSFMS (cm)              | 16.03\textsuperscript{c}  | 23.03\textsuperscript{b}  | 14.69\textsuperscript{b}  | 15.03\textsuperscript{b}  | 25.03\textsuperscript{b}  | 15.36\textsuperscript{b}  | <0.000  | 0.34312292 |
| LSFST (cm)              | 15.33\textsuperscript{c}  | 12.5\textsuperscript{a}  | 15.53\textsuperscript{b}  | 6.5\textsuperscript{a}  | 6.5\textsuperscript{a}  | 11.6\textsuperscript{a}  | <0.000  | 0.53683098 |
| Inflorescences emitted during the second flowering stage |     |
| Flowering date          |      |    |    |    |    |     |
| 6/03/20                 |      |    |    |    |    |     |
| 14/03/20                |      |    |    |    |    |     |
| 12/03/20                |      |    |    |    |    |     |
| 5/03/20                 |      |    |    |    |    |     |
| 27/03/20                |      |    |    |    |    |     |
| 23/03/20                |      |    |    |    |    |     |
| LSD value               |      |    |    |    |    |     |
| Inflorescences emitted during the third flowering stage |     |
| Flowering date          |      |    |    |    |    |     |
| 12/03/20                |      |    |    |    |    |     |
| 24/03/20                |      |    |    |    |    |     |
| 25/03/20                |      |    |    |    |    |     |
| 13/03/20                |      |    |    |    |    |     |
| 20/04/20                |      |    |    |    |    |     |
| 31/03/20                |      |    |    |    |    |     |
| LSD value               |      |    |    |    |    |     |
| Inflorescences emitted during the second flowering stage |     |

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*NS: Number of spathe. TLS: Total length of the spathe. TSW: Total spathe width. SW: Spathe weight. NSS: Number of spikelets per spathe. LLS: Length of longest spikelet. NFLS: Number of flowers per longest spikelet. SSL: Shortest spikelet length. NFSS: Number of flowers per shortest spikelet. LSWFTS: Length of the part of the spikelet without flower (at the basis of the spathe). LSWFTS: Length of the part of the spikelet without fruit (at the middle of the spathe). LSFBS: Length of the part of the spikelet without fruit (at the base of the spathe). LSFTS: Length of the part of the spikelet with flowers (at the middle of the spathe). LSFTS: Length of the part of the spikelet with flowers (at the top of the spathe).
TABLE III. LIST OF QUALITATIVE DESCRIPTORS OF THE INFLORESCENCE

| Pollinators Descriptors | ABD1 | P4 | P7 | P8 | P3 | P13 |
|-------------------------|------|----|----|----|----|-----|
| Inflorescences emitted during the first flowering stage |
| Flowering date          | 28/02/20 | 5/03/20 | 4/03/20 | 29/02/20 | 19/03/20 | 16/03/20 |
| SS                      | Lanceolate | Lanceolate | Swollen | Lanceolate | Fusiform | Lanceolate |
| SD                      | Cowardly | Densely | Medium | Medium | Medium | Cowardly |
| SPS                     | Simious | Straight | Very simious | Very simious | Simious | Very simious |
| Inflorescences emitted during the second flowering stage |
| Flowering date          | 6/03/20 | 14/03/20 | 12/03/20 | 5/03/20 | 27/03/20 | 23/03/20 |
| SS                      | Lanceolate | Lanceolate | Swollen | Lanceolate | Fusiform | Lanceolate |
| SD                      | Medium | Densely | Medium | Medium | Medium | Cowardly |
| SPS                     | Simious | Straight | Very simious | Simious | Very simious |
| Inflorescences emitted during the third flowering stage |
| Flowering date          | 12/03/20 | 24/03/20 | 25/03/20 | 13/03/20 | 20/04/20 | 31/03/20 |
| SS                      | Lanceolate | Lanceolate | Swollen | Lanceolate | Fusiform | Lanceolate |
| SD                      | Medium | Densely | Cowardly | Cowardly | Medium | Cowardly |
| SPS                     | Very simious | Straight | Very simious | Simious | Very simious |

*SS: Shape of the spathe, SD: Spikelet density, SPS: Spikelet shape.

Fig. 3. Dispersion of the three flowering stages (S1, S2 and S3, referring to early, medium, and late flowering stage, respectively) for each of the six male date palm genotypes in a plane defined by F1 and F2 axes of the principal component analysis (PCA) based on the morphological characteristics of the inflorescences.

B. Similarity Study

The application of the software MVSP version (3.22) to all of the morphological characteristics of inflorescences allowed us to obtain the matrix of the percentages of morphological similarities. Analysis of this matrix shows that the percentages of morphological similarity oscillate between 97.968% and 62.159% with an average of 80.062%, which testified to a strong morphological similarity within each genotype for early, medium, and late flowering stage and among the studied genotypes. The studied genotypes constitute convergent groups, especially since the indices of the percentages of morphological similarity are mostly close to 100%. The lowest coefficient (62.15%) was observed between the combinations ‘ABD1’ S3 and ‘P4’ S2. These coefficients reflect a low similarity in the morphological characters of these two genotypes, reflecting a great genetic diversity. On the other hand, the highest coefficient (97.968%) was recorded with the combinations of the genotypes ‘ABD1’ S1 and ABD1’S2 that resemble each other in a large number of characters, showing no morphological difference in spathe characteristics among the three flowering stages. According to Table II, the number of spathe developed per pollinator varies depending on flowering stage and cultivars. The total number of spathe varies from 30 to 36, with an average of 11 spathe per pollinator per flowering stage. This number reminds the average number of palms developed by cultivar per year [13], [14] indicating that the spathe develops in the axils of palms. The work of Behini [15] has shown that the number of spathe is little influenced by the year in almost all pollinators who have developed approximately the same number during the three years of measurement. Increases in the number of spathe can be influenced by irrigation disturbances [15]. Pollinator 13 was the one that recorded the maximum number of spathe developed (37), however, P3 was the one that gave the lowest number of spathe (30). The development of spathe can be late or early depending on the pollinator. Some pollinators begin to develop spathe in the autumn. The delay or precocity of the development of spathe in the same pollinator is influenced by the year [16]. In fact, the flowering of spathe constitutes one of the characteristics for the choice of pollinators. It owes its importance to the presence of pollen at the time of pollination. Before flowering, pollination of early date palms is only possible with pollen retained from the previous season, hence the need to select early flowering pollinators. In the sample tested, flowering of male spathe begins during the last week of February (ABD1) when certain varieties of date palm, mainly ‘white dates' such as the variety ‘Ammari' (the most early), are already receptive. According to Zaid and De Wet
flowering occurs when the temperature reaches over 18 °C. Flowering continues until the first week of April (P3). From the results of Table 1, the maximum flowering rate is recorded from the month of March (10th to 12th calendar week). According to Table 1, the analysis of variance shows a high significant difference between pollinators in terms of spathe length and according to flowering stage. These results indicate that the length of the spathe is under the predominance of genetic control. The variations recorded according to flowering stage are significant. From the results of Table I it can be seen that the maximum length of the spathe is recorded during the second flowering stage. The same results are recorded for the width of the spathe. Pollinators can be classified into three groups depending on the length of the spathe. Group with short spathe (P8 and P3) which length of the longest inflorescence is less than 1.12 m, group with medium spathe (ABD1) which length of the inflorescence is between 1.15 m and 1.30 m. The third group is formed by long spathe (P4, P7, and P13) which length of the longest spathe exceeds 1.50 m. These values are close to the intervals reported by Bhini [15] and which is between 80 and 150 cm, however they are greater than those of Babahani [16] and which are between 60 and 100 cm. According to Naser et al. [14], the length of the spathes is a good indicator of pollen quality; in fact, pollinators with large spathes have pollen grains with a metaxenic effect inducing the precocity of maturation [15]. The analysis of variance showed a significant variation in the number of spikelets per spathe and per flowering stage. From Table 1, we can see that the maximum number of spikelets is reached during the second flowering in all cultivars. The analysis of variance showed a significant variation in the number of spikes per spathe and per flowering stage. From Table 1 we can see that the maximum number of spikelets is reached during the second flowering in all cultivars. The maximum of spikelet is obtained with the pollinator P8 (450), while the minimum is recorded with the pollinator P13 (205). Traditionally, phoeniculturists use 1 to 3 male ears per female bunch [18]. The number of ears varies from 3 to 5 per bunch depending on the variety to be pollinated [1]. In addition, the number of spikelets produced per pollinator varies depending on the cultivar and flowering stage (table 1). The highest number of spikelets is recorded for cultivar P7 (427), while the lowest number is obtained for pollinator P13 (163) the maximum number of spikelets is obtained with the second flowering stage, these results are in agreement with those found by Rekis et al. [19]. The weight of the spadices oscillates between 1481 g and 3850 g, in fact, 22% of the spadices have less than 2000 g, and 45% have a weight between 2100 and 2700 g, while 33% concerns the spadices which weigh more than 3kg. These values are slightly higher than the intervals reported by Babahani [20] and Rekis et al. [19]. The results show that the length of the spikelets with flowers reaches the maximum in the second flowering stage, which therefore has a direct effect on the increase in pollen grain production. Concerning the shape of the spathe, 66% present the lanceolate form, 16% present the fusiform form and even 16% the swollen form (Table III), and these results are in agreement with those of Elkadri et al. [9]. From Table III we notice that the majority of qualitative characters are similar between the three flowering conditions only for the spikelet density character which varies from one stage to another for the two pollinators ABD1 and P7, these results are in agreement with those found by Rekis et al. [19] and Simozraget al. [21].

C. Phylogenetic Relationship

The similarity coefficients were processed by the NTYSYS software's WPGMA analysis program (version 2.02) to obtain a dendrogram that groups the different six male genotypes at each flowering stage/cycle (early, medium, and late) (Fig. 4). In this regard, two groups were distinguished. Group A contains the genotypes ‘P4’ and ‘P7’ for the three flowering stages. This group was divided into two subgroups, the first was composed of ‘P7’ and ‘P4’ at stage 2 while the second is subdivided into two sub-groups, one contains ‘P7’ at flowering stage 1 and 3 and the other consists of ‘P4’ at the early and late flowering stage of flowering. Group B is composed of pollinators ‘ABD1’, ‘P3’, ‘P8’ and ‘P13’ from the three flowering stages. This group is divided into two subgroups, the first consisting of ‘P13’ at various flowering stages and ‘ABD1’ at the late stage. The second subgroups included the remaining genotypes. Morphological studies of date palms have always been considered difficult to perform since they require a large set of phenotypic data and because date palms are quite diverse due to the environmental effect [22]. According to our results, it seems that the studied six male genotypes of date palm is characterized by a high level of genetic similarity of inflorescences at each flowering stage. This is strongly supported by projection of genotypes in the main PCA plane and in the analysis of WPGMA clusters.

![Dendrogram regroups of the six male date palm genotypes at various flowering stages (S1, S2 and S3, referring to early, medium, and late flowering stage/cycle, respectively) using morphological parameters of the inflorescences according to the WPGMA method of the NTSYS software.](image-url)

In our study, this discrimination between the studied six male genotypes could be analyzed based essentially on the following criteria: weight of the spathe (SW), number of spathe (NS), shape of spathe (SS), total length of the spathe (TLS), shape of spikelets (SPS), number of spikelets per spathe (NS), length of part of spikelet without fruit at base of spathe (LSWFS), number of flowers per shortest spikelet (NFSS) and length of part of spikelet with flower at base of spathe (LSFS). Morphological parameters have been used in several studies for the analysis of genotypes diversity in date palm. Al-Ghandiet al. [23] reported that the effectiveness of morphological traits associated with the inflorescence in differentiating between the male ‘Succary’
and other male genotypes. Iqbal et al. [24] evaluated and selected 15 elite male genotypes in Pakistan based mainly on the floral and morphological characteristics (length of the spike, number of flowers, etc.). Likewise, Soliman et al. [25] showed that the inflorescence characteristics were the most distinguishing factor of the male date palm ‘Succary’ from other genotypes. Kadri et al. [7] analyzed 38 Tunisian date palm male genotypes based mainly on the characters of the spikes and flowers than the characters of spathes and accordingly classified these males in three categories: good, medium, and bad. All these aspects showed the importance of the phenotypic study of the inflorescences and their great variability among date palm male genotypes. Several other studies have shown that morphological traits can be used to distinguish between date palm cultivars, including male genotypes ([7], [9], [24]-[29]). Our results confirm those of Bchini [15], who reported that the spathes morphological differences among different three flowering stages/cycles (early, medium, and late) during the flowering period were not significant in a Tunisian collection of 16 male date palm genotypes. In the current study, the various multi analyses of the inflorescence morphological parameters allowed to structure preliminary genetic relationships among the various stages of spathe emission of the studied six male date palm genotypes.

III. CONCLUSION

From the results obtained, we notice that there is no remarkable difference between the three flowering stages according to the qualitative characteristics of the inflorescences. However, in terms of quantity we notice that the second flowering stage is characterized by the most efficient sizes of the inflorescence (languor, width, weight, size, and number of spikeslets...), our results can be very useful for farmers when collecting inflorescences for pollination, in fact these results encourage farmers to use spathes from the second flowering to ensure a good pollination and a good yield of dates.

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Kadri Karim was born in Tunisia in 1978. He received the Diploma of engineering in Biotechnology from the National Institute of Applied Science and Technology (INSAT) in 2003 and the master’s degree on Genetic and molecular biology in 2005 and the PhD. degree in Biology in 2016 from the faculty of sciences of Tunisia. He joined the National Institute of agronomic research of Tunisia, in 2004 as an engineer. His task was morpho-physiological evaluation of barley accessions under salt stress. Since 2007, he has been affected in the Regional Research Center of Oasis Agriculture, as the university assistant, where he is currently the head of laboratory of biotechnology and genetic resources. His main areas of research interest are conservation and improvement of Date Palm genetic resources. His research activities are focused on the morphological, physiological, and molecular characterization of date palm cultivars in view of their preservation and conservation. Dr Kadri was interested by another filed of research, improving production efficiency in the date palm by the selection of metaxenic pollinators. Dr Kadri is the author and co-authored of many publications with Impact Factor, and over then 15 international articles as book chapters and proceedings of international congresses. He contributed to the organization of over 10 International congresses and courses in Tunisia and abroad and he reviewed papers for many International Journals. Dr. Kadri supervised many undergraduate and graduate students and developed collaborative projects bilateral research conventions. Dr. Kadri is a member of many scientific communities and structures, Laboratory of Biotechnology applied to agricultural (INRAT), the Tunisian Association of Genetic Resources (TAGR), the Tunisian Association of Biotechnology (ATB) and the Tunisia Dattes & Palm Cluster.