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RESEARCH ARTICLE

# Phenotypic and genetic characterization of date palm cultivars resistant to bayoud disease

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ABSTRACT

Taqerbucht cultivars of date palm are well known by their natural resistance against devastating fungus Bayoud disease. In order to know, if these accessions have the same genetic and morphological profile or each of them constitutes a separate cultivar, we carried out a morphological and molecular characterization and we compared four Taqerbucht (Tq.) date palm cultivars from the southwestern region of Algeria: Tq.hamra cultivar (red fruits), Tq. safra cultivar (yellow fruits), Tq.beïda (white fruits) and Tq.kahla cultivar (black fruits). Seventy one phenotypic characteristics, including 33 quantitative and 38 qualitative traits, have been selected for comparison. Principal component analysis (PCA) and multi-component clustering were used to analyze and compare the data. The results suggest that the four cultivars can be classified into distinct groups. One group contains one cultivar, the Tq.kahla and another group contains the three other cultivars (Tq.safra, Tq.beïda and Tq. hamra). Based on phylogenetic analyses and sequence comparisons, the cultivar Tq. kahla seems to be divergent from the cultivar Tq.hamra, whereas the two cultivars Tq.Safra and Tq.beïda are close to each other. Using 16 Simple Sequence Repeat (SSR) genetic markers to analyze genetic diversity among the cultivars, we found that 13 markers were detectable in 31 allele's loci, and the number of alleles per locus varied from 1–4 with an average of 2.38 alleles per locus. Expected heterozygosity (He) values ranged from 0.375–0.500 and observed heterozygosity (Ho) values from 0.750–1.000.

## Introduction

Date palm is an important staple food, financial and income source of millions of Saharan people in Africa and Asia. Algerian oases are home to the most important date palm genetic resources in North Africa, with about 18.5 million palm trees and 1100 cultivars, covering about 169,380 ha (1, 2). The Adrar region (southwest of Algeria), where this study was performed, has more than 400 cultivars, and the most common ones are Tilemsou, Tinasser Taqerbucht and Ahartane.

Palm traits are greatly influenced by environmental conditions and developmental stages (3). Palm genetic diversity is also endangered by multiple biotic factors, such as the bayoud disease (*Fusarium* wilt) caused by a telluric fungus called *Fusarium oxysporum* f. sp. *albedinis*, which has ravaged about 3 million date palm trees in Algeria (4). All attempts to control this scourge have been unsuccessful (5, 6). Various control measures have also been used to counteract the general effects of bayoud, such as improving farming practices, biological and chemical applications and genetic control techniques. Only the Taqerbucht cultivar has a natural resistance against this devastating fungus (7).

Furthermore, genetic control, the use of resistant cultivars, remains the most promising and least toxic to the environment. The generalized resistance of cultivar Taqerbucht to bayoud is remarkable in the oases of Touat, Gourara and Tidikelt in the South of Algeria. It is a cultivar that is restricted to the western regions. Thus, the preservation and multiplication of such a genetic resource are of great importance for date palm farmers to breed new resistant cultivars to reduce yield losse and increase date palm quality (8).

Several methods based on genetic, morphological and molecular analyses can be used to achieve this goal. Date palms can multiply by two main methods. The first is vegetative propagation representing about 10% of palm populations in Algeria. The second is

propagation by seeds, a widely used method for breeding new palm variety with valuable genetic and organoleptic qualities (9, 10). Morphological characteristics such as shape, size, weight, colour, the appearance of the fruit epidermis, fruit consistency, texture etc. are important traits to consider in breeding programmes. Physiological and biochemical characterization, such as the content and types of flavours and flavonoids produced by acid hydrolysis, can also be helpful for the taxonomy and classification of date palm cultivars (11). DNA-based markers also provide valuable information on genetic diversity and relationship between cultivars at molecular levels to identify kinship links and other characteristics that are difficult to figure out by morphological and biochemical analysis. For example, Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat (ISSR), Random Amplified Microsatellite Polymorphism (RAMP) have been used for the characterization of germplasm of different date palm cultivars from Saudi Arabia, Qatar, Egypt, Tunisia, Sudan, Mauritania and Morocco with similar climatic conditions (12–14). Recently, 17 date palm microsatellite markers have been identified using $(GA)_n$ and $(GT)_n$ repeats (15). Microsatellites and Simple Sequence Repeats (SSRs) have also proven to be helpful in a wide range of applications in genetics (16) as they are relatively easily amplifiable by PCR (Polymerase Chain Reaction), their co-dominant nature and their generally high level of allelic diversity. Twenty-eight microsatellites have already been used to analyze phylogenetic relationships among Iranian and Spanish date palm varieties (17).

However, Little works have been performed so far to phenotypically characterize Taqerbucht cultivars in Algeria, especially in regard to resistance properties against bayoud disease. In this study, our goal was to explore genetic diversity within four date palm cultivars within the variety 'Taqerbucht' using nuclear microsatellite markers. We aimed at unravelling genetic relationships and comparing their morphometric traits to identify relevant characteristics that can be used as descriptors in the field.

## Materials and Methods

### *Botanical and morphological sampling*

Date palm samples from four Taqerbucht cultivars named "Taqerbucht safra", "Taqerbucht hamra", "Taqerbucht Beïda" and "Taqerbucht Kahla" (Table 1) were collected from trees growing in two oases located in Adrar region of Algeria south-west.

The first is Adrar oasis located at 279 m altitude, 27.84°N latitude and 0.33°W longitudes and the second is Aougrout oasis located at 281 altitude, 28.70°N latitude, and 0.30°W longitude (Fig. 1).

The accessions were chosen for the quality of their fruits and the importance in the socio-economic life of native people of the region and resistance to *Fusarium oxysporum* f. sp. *albedinis*. The Taqerbucht cultivars underwent a comprehensive morphological study on the growth of stock, palm, brunch, fruit and seed.

A total of 71 descriptors, quantitative and qualitative, adopted by the International Plant Genetic Resources Institute (IPGRI) have been used (Table 2, 3) to compare the four cultivars in terms of descriptor stability and resistance to Bayoud disease.

### *Molecular analysis*

In order to characterize the accessions of Taqerbucht, sixteen SSR have been used (16) (Table 4).

Leaflets of juvenile palms from the middle crown of adult palms aged about 15 years from the four date palm cultivars were used for molecular analyses. Genomic DNA was extracted from the leaflets using a NucleoSpin® Plant II extraction kit (Macherey-Nagel, Inc). PCR reactions have been performed in 96-well microplates containing 20 µl reaction mixtures composed of 100 ng genomic DNA, 1X Taq buffer, 0.5U DNA Taq polymerase (Promega), MgCl (1.5 mM), dNTP (0,2 mM each), 50 pmol each Forward and Reverse primer. A PCR contamination control without genomic DNA was used to ensure the absence of contamination. The amplification PCR program was the following: 95 °C for 5 min (initial denaturation), followed by 35 cycles of 95 °C for the 30 seconds, 56 °C for 30 seconds (primer hybridization) and 72 °C for the 30 seconds (primer extension), with a final extension phase at 72 °C for 10 min. PCR products were then separated on 1% agarose gel Tris, Borate, EDTA (TBE) to check amplification quality. PCR amplification reactions were then kept at 4 °C until use. Ten microliters (10µl) of each PCR amplification product (SSR markers) were then separated by electrophoresis (vertical gel electrophoresis system) in 8 % non-denaturing acrylamide gel along with a DNA ladder (Hyper Ladder V) at 250 Volt and 170 mA for 150 minutes. The gels were then visualized under UV transilluminator and DNA bands photographed.

### *Statistical analysis*

To compare intra-cultivar variations, 33 quantitative morphological variables (Table 5) were analyzed separately by ANOVA test with posthoc LSD using Statistica software (19). Principal component analysis (PCA) and multi-component analysis (MCA) have been used to analyze and compare the measures of morphological parameters of growth, palms, inflorescences, fruits and seeds to determine the characteristics for variability using XLSTAT 2004.

In addition, the expected heterozygosity (He), the Hardy–Weinberg equilibrium and the null allele frequencies were calculated using GenAlEx6.5 software (New Brunswick, NJ) (20) and CERVUS 3.0.3 (Bozeman, MT) (21, 22). Genetic distance dendrograms have been established using MEGA 6 software (23) according to Nei's minimum genetic distance matrix (24). The bootstrap resampling methodology (1000 replicates) was performed to test the robustness of the dendrogram topology. Wright's F-statistics (FIT, FIS, FST) (25, 26) were calculated with POPGENE (27).

**Table 1.** Phenotypic characterization of the four Taqerbucht fruit cultivars (black, red, white and yellow)

| Variety Name | Code (short name) | Dates colour | Precocity | Appreciation by consumers |
|---|---|---|---|---|
| Taqerbucht safra | Tq. safra | Yellow | Late | average |
| Taqerbucht beïda | Tq. beïda | White | late | average |
| Taqerbucht hamra | Tq. hamra | Red | late | average |
| Taqerbucht kahla | Tq. kahla | black | semi-late | good |

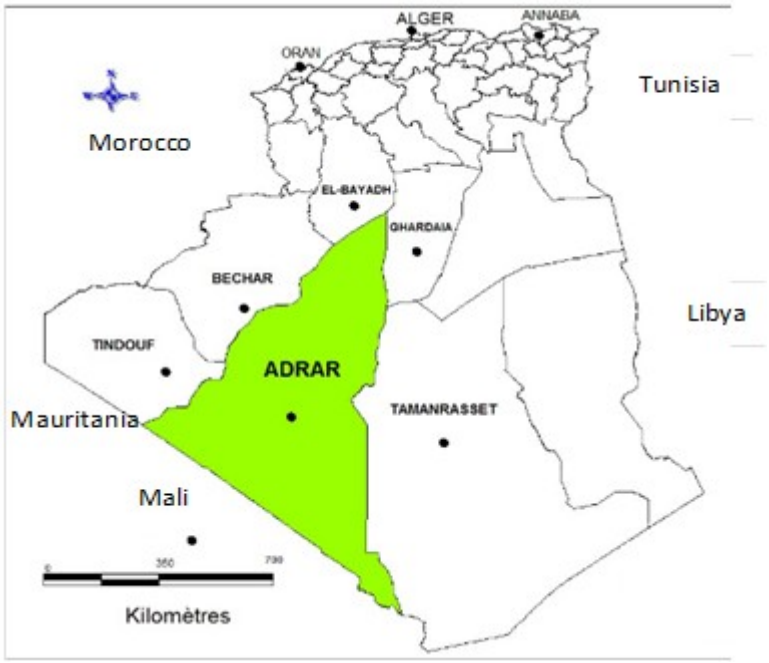


**Fig. 1.** Location of the study area (18).

**Table 2.** Morphometric comparative characteristics between four cultivars of the Taqerbucht date variety. Thirty-three quantitative characters were used in the comparison

| Characters | | Quantitative character | character code |
|---|---|---|---|
| 1 | **Palm** | Palm length (cm) | PAL |
| 2 | | Palm width (cm) | PAW |
| 3 | | Spines number | SN |
| 4 | | Spines thickness | ST |
| 5 | | Spines length (cm) | SL |
| 6 | | Pinna number | PN |
| 7 | | Pinna width | PIW |
| 8 | | Pinna length | PIL |
| 9 | | Spacing index | SI |
| 10 | **Inflorescence** | Spathe length | SPL |
| 11 | | Spathe width | SPW |
| 12 | | Stem length | STL |
| 13 | | Stem width | STW |
| 14 | | Thickness Stem | TST |
| 15 | | Spikelet Numbers per diet | SPND |
| 16 | | Longest spikelet | LSP |
| 17 | | Shortest spikelet | SSP |
| 18 | | Spikelet with flower base | SPF |
| 19 | | Spikelet length with flower in middle | SPLFM |
| 20 | | Spikelet length with flower at the top | SPLFT |
| 21 | | Flower Numbers per longest spikelet | FNLSP |
| 22 | | Flower numbers per shortest spikelet | FNSSP |
| 23 | | N° of flowers knotted/spikelet at base | NFNKSP |
| 24 | **Fruit** | Fruit weight | FW |
| 25 | | Flesh thickness | FT |
| 26 | | Cavity length | CL |
| 27 | | Cavity width | CW |
| 28 | | Chalice diameter | CD |

| | | | |
|---|---|---|---|
| 29 | **Seed** | Length of seed | LSD |
| 30 | | Width of seed | WSD |
| 31 | | Seed thickness | SDT |
| 32 | | Seed weight | SDW |
| 33 | | Weight of seed/to fruit | WSDF |

**Table 3.** Morphometric comparative characteristics between four cultivars of the Taqerbucht date variety. Thirty-eight qualitative characters were used in the comparison

| | | **Qualitative characters** | **character code** |
|---|---|---|---|
| 01 | **Palm** | Appearance of Middle Crown | AMC |
| 02 | | Presence of Air Off-Shoots | PAOS |
| 03 | | Density of Fibrillium | DF |
| 04 | | Hardness of Fibrillium | HF |
| 05 | | Ability To Produce Off Shoots | APOS |
| 06 | | Palm curvature Level | PACL |
| 07 | | Angle of The Palm | APA |
| 08 | | Rotation of The Palm | RPA |
| 09 | | Petiole color | PTC |
| 10 | | Penne color | PNC |
| 11 | | Disposition of Pinna | DIP |
| 12 | | Apical divergence Pinna | ADPI |
| 13 | **Inflorescence** | Spathe form | SF |
| 14 | | Diet position | DP |
| 15 | | Stem color | STC |
| 16 | | Density of Spikelets | DSP |
| 17 | | Form of Spikelets | FSP |
| 18 | **Fruit** | Fruit form | FF |
| 19 | | Fruit color | FC |
| 20 | | Fruit form at base | FFB |
| 21 | | fruit form at the top | FFS |
| 22 | | Fruit length at Bser stage | LFB |
| 23 | | fruit width at Bser stage | FWBS |
| 24 | | fruit color at tamer stage | FWTS |
| 25 | | Flesh texture | FT |
| 26 | | Fruit taste | FTA |
| 27 | | Chalice form | CF |
| 28 | | Chalice color | CC |
| 29 | **Seed** | Seed shape | SS |
| 30 | | Seed length/Fruit | SLF |
| 31 | | Seed color | SEC |
| 32 | | Surface aspect | SA |
| 33 | | Furrow form of seed | FFS |
| 34 | | Germ pore situation | GPS |
| 35 | | Protuberance type | PT |
| 36 | | protuberance Frequency | PF |
| 37 | | Mucron presence | MP |
| 38 | | Tegument adhesion | TA |

**Table 4.** Sixteen Short Sequence Repeats (SSR) isolated from *Phoenix dactylifera* used in the comparison of four date cultivars (Tq. beïda, Tq. hamra, Tq. kahla and Tq. safra) using PCR oligonucleotides as described previously

| **SSR locus** | **EMBL Accession no.** | **Repeat motif** | **Clone size (bp)** | **Primer sequences (5'–3')** | **Optimal Ta (°C)** |
|---|---|---|---|---|---|
| mPdCIR010 | AJ571673 | $(GA)_{22}$ | 180 | F: ACCCCGGACGTGAGGTG<br>R: CGTCGATCTCCTCCTTTGTCTC | 55.9 |
| mPdCIR015 | AJ571674 | $(GA)_{15}$ | 253 | F: AGCTGGCTCCTCCCTTCTTA<br>R: GCTCGGTTGGACTTGTTCT | 51.6 |
| mPdCIR016 | AJ571675 | $(GA)_{14}$ | 209 | F: AGCGGGAAATGAAAAGGTAT<br>R: ATGAAAACGTGCCAAATGTC | 51.7 |
| mPdCIR025 | AJ571676 | $(GA)_{22}$ | 269 | F: GCACGAGAAGGCTTATAGT<br>R: CCCCTCATTAGGATTCTAC | 49.3 |
| mPdCIR032 | AJ571677 | $(GA)_{19}$ | 376 | F: CAAATCTTTGCCGTGAG<br>R: GGTGTGGAGTAATCATGTAGTAG | 51.5 |
| mPdCIR035 | AJ571678 | $(GA)_{15}$ | 341 | F: ACAAACGGCGATGGGATTAC<br>R: CCGCAGCTCACCTCTTCTAT | 53.9 |

| | | | | |
|---|---|---|---|---|
| mPdCIR044 | AJ571679 | $(GA)_{19}$ | 340 | F: ATGCGGACTACACTATTCTAC<br>R: GGTGATTGACTTTCTTTGAG | 51.7 |
| mPdCIR048 | AJ571680 | $(GA)_{32}$ | 439 | F: CGAGACCTACCTTCAACAAA<br>R: CCACCAACCAAATCAAACAC | 51.4 |
| mPdCIR050 | AJ571681 | $(GA)_{21}$ | 568 | F: CTGCCATTTCTTCTGAC<br>R: CACCATGCACAAAAATG | 48.5 |
| mPdCIR057 | AJ571682 | $(GA)_{20}$ | 360 | F: AAGCAGCAGCCCTTCCGTAG<br>R: GTTCTCACTCGCCCAAAAATAC | 55.4 |
| mPdCIR063 | AJ571683 | $(GA)_{17}$ | 301 | F: CTTTTATGTGGTCTGAGAGA<br>R: TCTCTGATCTTGGGTTCTGT | 49.8 |
| mPdCIR070 | AJ571684 | $(GA)_{17}$ | 265 | F: CAAGACCCAAGGCTAAC<br>R: GGAGGTGGCTTTGTAGTAT | 48.7 |
| mPdCIR078 | AJ571685 | $(GA)_{13}$ | 260 | F: TGGATTTCCATTGTGAG<br>R: CCCGAAGAGACGCTATT | 49.6 |
| mPdCIR085 | AJ571686 | $(GA)_{29}$ | 375 | F: GAGAGAGGGTGGTGTTATT<br>R: TTCATCCAGAACCACAGTA | 50.4 |
| mPdCIR090 | AJ571687 | $(GA)_{26}$ | 269 | F: GCAGTCAGTCCCTCATA<br>R: TGCTTGTAGCCCTTCAG | 48.6 |
| mPdCIR093 | AJ571688 | $(GA)_{16}$ | 230 | F: CCATTTATCATTCCCTCTCTTG<br>R: CTTGGTAGCTGCGTTTCTTG | 51.8 |

**Table 5.** Descriptive statistics of four date cultivars (Tq. kahla, Tq. safra, Tq. hamra and Tq. beïda). **SD**: Standard deviation, * significant ($p<0.05$); ns: significant

| | Trait code | Tq. KAHLA | | Tq. SAFRA | | Tq. HAMRA | | Tq. BAÏDA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | *P-value* | Mean of means |
| Palm | PAL | 337.3 | 7.11 | 351.7 | 1.56 | 384.7 | 0.44 | 315.7 | 0.44 | *0.36 ns* | 347.35 |
| | PAW | 70.7 | 0.89 | 68.3 | 2.22 | 83.7 | 2.89 | 86.7 | 0.44 | *0.89 ns* | 77.35 |
| | SN | 47.3 | 1.11 | 41 | 2.67 | 44 | 0.67 | 36.7 | 1.11 | *0.02** | 42.25 |
| | ST | 7.3 | 0.44 | 7 | 0.04 | 8.2 | 0.22 | 9 | 0.18 | *0.24 ns* | 7.875 |
| | SL | 13.5 | 1.01 | 10.15 | 0.27 | 18.8 | 0.56 | 12.7 | 0.18 | *0.91 ns* | 13.7875 |
| | PN | 184 | 3.33 | 171.7 | 4.22 | 190 | 1.33 | 141.3 | 6.44 | *0.11 ns* | 171.75 |
| | PIW | 34.7 | 2.22 | 36.25 | 0.33 | 22.8 | 0.56 | 25.3 | 1.11 | *0.42 ns* | 29.7625 |
| | PIL | 454.2 | 9.44 | 444.2 | 22.78 | 420 | 0.67 | 562.7 | 1.56 | *0.28ns* | 470.275 |
| | SI | 0.36 | 0 | 0.46 | 0 | 0.63 | 0 | 0.81 | 0.03 | *0.49ns* | 0.565 |
| | | 127.71* | 2.84 | 125.64* | 3.79 | 130.31* | 0.82 | 132.32* | 1.28 | | |
| Inflorescence | SPL | 58.33 | 2.22 | 67 | 7.33 | 64.5 | 0.33 | 68.33 | 1.11 | *0.49ns* | 64.54 |
| | SPW | 9.4 | 0.42 | 9.9 | 0.09 | 10.5 | 0.67 | 8.1 | 0.11 | *0.00** | 9.475 |
| | STL | 154.7 | 3.56 | 131 | 12 | 165 | 10 | 133 | 12 | *0.004** | 145.925 |
| | STW | 46.3 | 1.56 | 41.3 | 2.44 | 91.7 | 5.56 | 54.3 | 8.89 | *0.000** | 58.4 |
| | TST | 22.67 | 0.44 | 17.67 | 1.78 | 28 | 2 | 23 | 2 | *0.000** | 22.835 |
| | SPND | 58.67 | 0.44 | 90 | 0.67 | 100.67 | 9.56 | 136 | 2 | *0.000** | 96.335 |
| | LSP | 55 | 4.67 | 42 | 2 | 65.67 | 5.11 | 52.33 | 2.22 | *0.000** | 53.75 |
| | SSP | 30 | 2 | 22.67 | 0.89 | 22.67 | 3.56 | 20.5 | 1 | *0.000** | 23.96 |
| | SPF | 35.5 | 1.78 | 26 | 1.78 | 36.67 | 2.22 | 29.83 | 1.67 | *0.000** | 32 |
| | SPLFM | 27.17 | 1.89 | 22.83 | 2.56 | 25 | 2 | 26.17 | 0.56 | *0.000** | 25.2925 |
| | SPLFT | 20.5 | 2.33 | 18.67 | 3.44 | 18 | 0.67 | 16.5 | 0.33 | *0.002** | 18.4175 |
| | FNLSP | 45.33 | 1.11 | 39 | 1.33 | 39 | 0.67 | 44.67 | 3.56 | *0.001** | 42 |
| | FNSSP | 33.67 | 3.56 | 25 | 4.67 | 24.67 | 1.78 | 30.33 | 2.44 | *0.021** | 28.4175 |
| | NFNKSP | 11 | 1.33 | 10 | 1.33 | 11.67 | 2.44 | 17.33 | 0.89 | *0.000** | 12.5 |
| | | 43.45* | 1.95 | 40.22* | 3.02 | 50.27* | 3.33 | 47.17* | 2.77 | | |
| Fruit | FW | 12.23 | 0.37 | 11.07 | 0.97 | 15.13 | 0.4 | 10.85 | 0.59 | *0.000** | 12.32 |
| | FT | 8 | 0.67 | 6.7 | 0.44 | 4.8 | 0.56 | 6 | 1.33 | *0.000** | 6.375 |
| | CL | 26.7 | 0.44 | 26.8 | 1.22 | 31.7 | 2.22 | 29.3 | 0.89 | *0.005** | 28.625 |
| | CW | 16.7 | 1.11 | 13.3 | 0.44 | 13.2 | 0.89 | 12.3 | 0.89 | *0.001** | 13.875 |
| | CD | 9.8 | 0.56 | 12 | 0 | 10.8 | 0.56 | 11.3 | 0.44 | *0.001** | 10.975 |
| | | 14.68* | 0.63 | 13.97* | 0.614 | 15.13* | 0.93 | 13.95* | 0.83 | | |
| Seed | LSD | 22.67 | 0.44 | 24 | 1.33 | 24.67 | 1.11 | 21 | 2 | *0.01** | 23.085 |
| | WSD | 10.17 | 0.56 | 9 | 0 | 8.83 | 0.22 | 8.67 | 0.44 | *0.000** | 9.1675 |
| | SDT | 8.67 | 1.11 | 8.67 | 0.89 | 8 | 0 | 8 | 0 | *0.704ns* | 8.335 |
| | SDW | 1.4 | 0.02 | 1.3 | 0.12 | 1.2 | 0.04 | 1.3 | 0.11 | *0.378ns* | 1.3 |
| | **WSDF** | 0.13 | 0.018 | 0.12 | 0.01 | 0.08 | 0.02 | 0.1 | 0.01 | *0.000** | 0.1075 |
| | | 8.61* | 0.43 | 8.62* | 0.47 | 8.56* | 0.28 | 7.81* | 0.51 | *0* | |

## Results

### *Morphological analysis*

Thirty three quantitative and 38 qualitative traits (Table 2, 3) in seeds, fruits, palm and inflorescence (flowers) have been compared between four date cultivars from the variety Taqerbucht (Tq). The four cultivars are Tq. beïda (a date palm variety with white fruits), Tq. hamra (a variety with red fruits), Tq. kahla (a variety with black fruits) and Tq. safra (a variety with yellow fruits. Fruit maturities in these cultivars are relatively late, and their appreciation by consumers varies from average to good. While consumers averagely appreciate yellow, white and red fruits, black fruit (Tq. kahla), on the other hand, is highly appreciated for their morphological and physiological properties (shape, colour and taste) where they are sweeter than other varieties.

Inter-varietal comparison between the four Taqerbucht cultivars show that about 11 qualitative characteristics, mostly in the palm organ, were relatively conserved or similar among the four cultivars.

The comparison of the total quantitative and qualitative traits (a sum of 71 traits) between the four cultivars (Tq. beïda, Tq. hamra, Tq. kahla, and Tq. safra) show four distinct groups as revealed by a principal component analysis (Fig. 2). The phenotypic characterization of the two cultivars Tq. beïda and Tq. safra seems to be grouped into one large related group.

Based on the 38 qualitative morphological traits, the four cultivars were grouped into nine distinct groups according to common/distinctive traits as shown by a multi-component analysis (Fig. 3). The variety Tq. kahla comprised four groups (groups 1, 2, 3 and 4), the variety Tq. hamra contains two groups (groups 6 and 7) while the two cultivars Tq. safra and Tq. beïda shared three groups of common characteristics (groups 5, 8 and 9).

### *Molecular and polymorphism analysis*

A total of 16 Short Sequence Repeat (SSR) markers have been used for molecular and polymorphism analyses between the four Taqerbucht cultivars. We, however, could not obtain amplification for three SSR markers (mPdCIR044, mPdCIR063 and mPdCIR070). Hence, we removed them from the analysis and used the remaining 13 SSR markers in which the marker mPdCI057 seemed to be monomorphic (Table 6).

Using these markers, 31 alleles were detectable in 13 loci. The number of alleles per locus varied from one mPdCIR057 to four mPdCIR090 with a mean of 2.38 alleles per locus. The effective mean number of alleles was 1.86. Expected heterozygosity (*He*) values ranged from 0.375 (mPdCIR025 and mPdCIR078) to 0.500 (the rest of all loci). The lowest and highest observed heterozygosity (*Ho*) values were 0.750 (mPdCIR025 and mPdCIR078) and 1.0, respectively, for the other loci. For all markers, the values of observed heterozygosity (*Ho*) were higher than expected. In order to analyze the stability of expected heterozygosity in the studied populations, we performed the Wright F-statistics (Fis, Fit, Fst) (25). In fact, The Fis values were negative for all markers with an average of -1.0 (Table 6). *Fst* values varied between 0 for six markers to 0.520 for the mPdCIR078 marker with an average of 0.122. All markers, except two (mPdCIR025 and mPdCIR078), showed a significant *HW* deviation.

### *Intra-cultivars genetic diversity*

The number of alleles per variety ranged from 23 for the cultivar Tq. safra to 26 for the cultivar Tq. hamra with a mean value of 24.5. The two cultivars Tq. hamra and Tq. beïda showed the highest values in terms of mean heterozygosity observed. The expected heterozygosity values in the four cultivars were less than the observed values. The *Fis* value, an important factor in defining population structure and indicating heterozygosity loss, ranged from -1.0 for the cultivar Tq. hamra to -0.692 for the cultivar Tq. kahla. Only seven alleles have been identified as specific to the

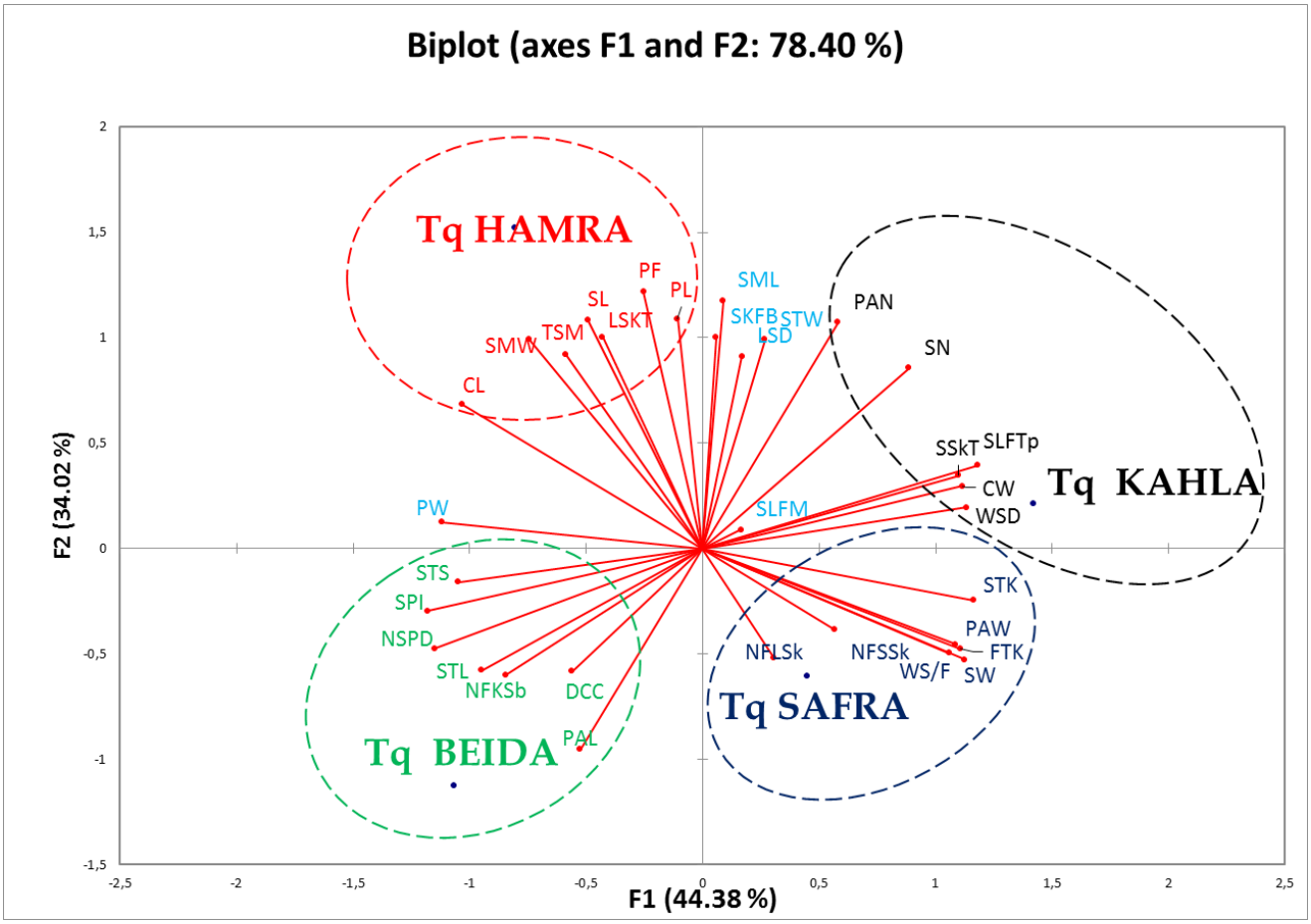


**Fig. 2.** PCA. Representation of accessions and quantitative morphological characteristics (Biplot).

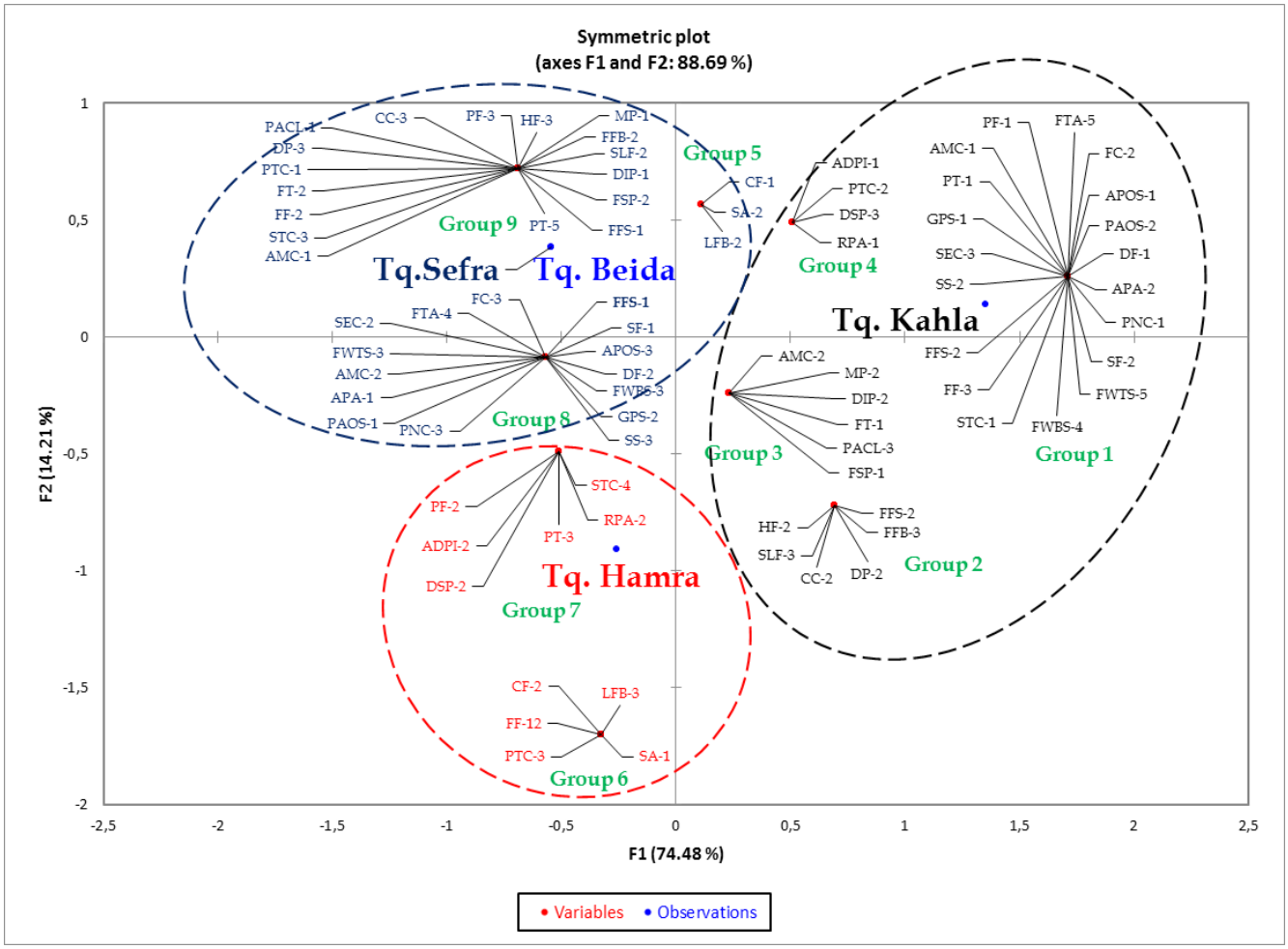


**Fig. 3.** MCA representation of cultivars and qualitative morphological characters.

**Table 6.** Polymorphisms of 13 microsatellites used to compare four date cultivars

| Loci | NA | Ne | Ho | He | F | Fis | Fit | Fst | Nm | EHW |
|---|---|---|---|---|---|---|---|---|---|---|
| mPdCIR010 | 3 | 2 | 1 | 0.5 | -1.000 | -1.000 | -0.684 | 0.158 | 1.333 | * |
| mPdCIR015 | 2 | 2 | 1 | 0.5 | -1.000 | -1.000 | -1 | 0 | / | ** |
| mPdCIR016 | 2 | 2 | 1 | 0.5 | -1.000 | -1.000 | -1 | 0 | / | ** |
| mPdCIR025 | 2 | 1.75 | 0.75 | 0.375 | -1.000 | -1.000 | -0.6 | 0.2 | 1 | Ns |
| mPdCIR032 | 2 | 2 | 1 | 0.5 | -1.000 | -1.000 | -1 | 0 | / | ** |
| mPdCIR035 | 3 | 2 | 1 | 0.5 | -1.000 | -1.000 | -0.684 | 0.158 | 1.333 | * |
| mPdCIR048 | 2 | 2 | 1 | 0.5 | -1.000 | -1.000 | -1 | 0 | / | ** |
| mPdCIR050 | 2 | 2 | 1 | 0.5 | -1.000 | -1.000 | -1 | 0 | / | ** |
| mPdCIR057 | 1 | 1 | 0 | 0 | / | / | / | / | / | Monomorphic |
| mPdCIR078 | 3 | 1.5 | 0.75 | 0.375 | -1.000 | -1.000 | 0.04 | 0.52 | 0.231 | ns |
| mPdCIR085 | 2 | 2 | 1 | 0.5 | -1.000 | -1.000 | -1 | 0 | / | ** |
| mPdCIR090 | 4 | 2 | 1 | 0.5 | -1.000 | -1.000 | -0.455 | 0.273 | 0.667 | ** |
| mPdCIR093 | 3 | 2 | 1 | 0.5 | -1.000 | -1.000 | -0.684 | 0.158 | 1.333 | * |
| **Mean** | 2.385 | 1.865 | 0.885 | 0.442 | -1.000 | -1.000 | -0.756 | 0.122 | 0.454 | |

**Na** = Number of Different Alleles; **Ne** = N° of Effective Alleles = 1 / (Sum pi^2); I = Shannon's Information Index = -1* Sum (pi * Ln (pi)); Ho = Observed Heterozygosity = N° of Hets / N ; Na = No. of Different Alleles; Ne = No. of Effective Alleles = 1 / (Sum pi^2); I = Shannon's Information Index = -1* Sum (pi * Ln (pi)); **Ho** = Observed Heterozygosity = N° of Hets / N ; **He** = Expected Heterozygosity = 1 - Sum pi^2, **F** = Fixation Index = (He - Ho) / He = 1 - (Ho / He), **Fis , Fst, Fit** (Fstatistic).

cultivar Tq. kahla and the percentage of polymorphic loci per cultivar varied from 84.62–92.31 %, with an average of 88.46 % (Table 7).

The level of genetic differentiation among cultivars was analyzed by ANOVA, and it was found that the percentage of molecular variability in the four cultivars were about 92 % within individuals and 8 % among populations (Fig. 4). This suggests that the cultivars are homogeneous, and the differentiation is more individual than population-related. This is probably due to plantation errors and farmers' practices mixing between varieties.

On the other hand, in the phylogenic tree constructed using Nei's minimum genetic distance (Fig. 5), the cultivar Tq. kahla appears a bit distant from the others. Pairwise comparisons between populations confirm this distance, where the cultivar Tq. kahla has seven specific alleles (Table 7). A solid

correlation, however, between the two cultivars Tq. hamra and Tq. beïda (r = 1) was noticeable (Table 8).

To visualize the relationship between individuals from different cultivars and to figure out any possible admixtures between populations, a factorial correspondence analysis (FCA) was performed using GENETIX4.05 software. About 88.47 % of the total variations distinguish the cultivar Tq. kahla from the other cultivars. By contrast, the second axis representing 10.47 % of the total variation showed the isolation of Tq. safra, Tq. hamra and Tq. beïda (Fig. 6).

**Table 7.** Intra-cultivar genetic diversity

| Cultivars | Na | Ne | Ho | He | F | Fis | specific alleles ≥ 50% | HW | %P |
|---|---|---|---|---|---|---|---|---|---|
| Tq. Safra | 23 | 1.769 | 0.846 | 0.423 | -1.000 | -0.833 | / | NS | 84.62% |
| Tq. Hamra | 26 | 1.923 | 0.923 | 0.462 | -1.000 | -1.000 | / | Ns | 92.31% |
| Tq. Beïda | 25 | 1.923 | 0.923 | 0.462 | -1.000 | -0.846 | / | NS | 92.31% |
| Tq. Kahla | 24 | 1.846 | 0.846 | 0.423 | -1.000 | -0.692 | 7 | Ns | 84.62% |
| **Mean** | 24.5 | 1.865 | 0.885 | 0.442 | -1.000 | -0.842 | | | 88,46% |

**Na**: N° of different alleles, **Ne**: No. of effective alleles: 1 / (Sum pi^2), **Ho**: Observed Heterozygosity; No of Hets / N, **He**: Expected Heterozygosity = 1 - Sum pi^2, **F** = Fixation Index = (He - Ho) / He = 1 - (Ho / He), **Fis , Fst, Fit** (Fstatistic), Nm = ((1 / Fst) - 1) / 4, ns=not significant, P%: percentage of polymorphic loci.

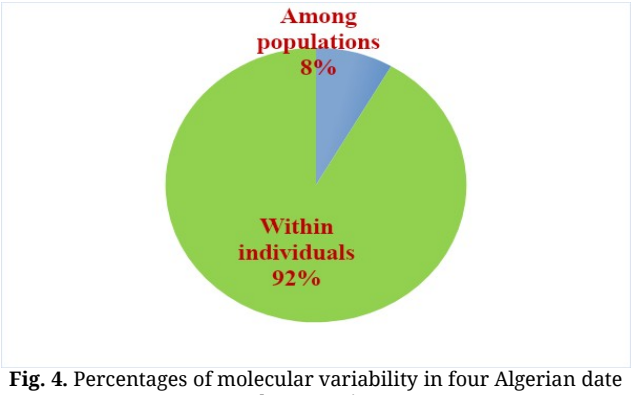


**Fig. 4.** Percentages of molecular variability in four Algerian date Palm accessions.

## Discussion

We aimed to phenotypically and genetically compare these cultivars to deduce relationships between them in terms of morphological and genetic characteristics involved in environmental, economic and nutritive values. Using statistical analyses, we observed some highly significant intra-varietal variations that can be classified into two distinct types: 1) those that are conserved or common between the four cultivars, and 2) those specific or variable between the cultivars. The first type of conserved characteristics seems to be related to vegetative organs, mainly the palm, while the second category of traits seems to be linked to the reproductive organs, particularly inflorescence and seeds.

Multivariate analyses using 33 quantitative traits identified four groups, almost one group for each variety. These groups, however, share well-defined quantitative and morphological characteristics. For example, the black-fruit variety (Tq. kahla) is positively correlated with quantifiable morphological traits such as the spikelet length with flowers at the top (SPLFT) (r = 0.9), shortest spikelet (SSP) (r = 0.78), cavity width (CW) (r = 0.80), the width of the seed (WSD) (r = 0.82), and Spines number (SN) (r = 0.51), suggesting that these traits are conserved. The red-fruit cultivar (Tq. hamra) was characterized by the following quantitative traits: fruit weight (FW) (r = 0.95), palm length (PAL) (r = 0.76), long spikelet (LSP) (r = 0.65), spine length (SL) (r = 0.76), thickness stem (inflorescence carrier) (TST) (r = 0.54) and stem width

**Table 8.** Pairwise Population Matrix according to Nei's Genetic Identity

| | Tq. safra | Tq. hamra | Tq. beïda | Tq. kahla |
|---|---|---|---|---|
| Tq. Safra | 1.000 | | | |
| Tq. hamra | 0.964 | 1.000 | | |
| Tq. Beïda | 0.964 | 1.000 | 1.000 | |
| Tq. Kahla | 0.752 | 0.759 | 0.759 | 1.000 |

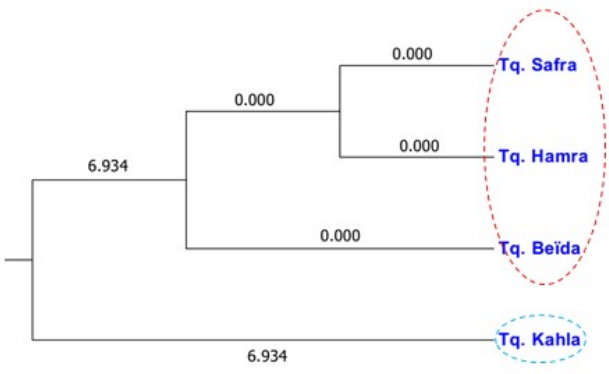


**Fig. 5.** Dendrogram based on Nei's (1972) genetic distance: Method = UPGMA of 4 cultivars of palm date in Algeria.

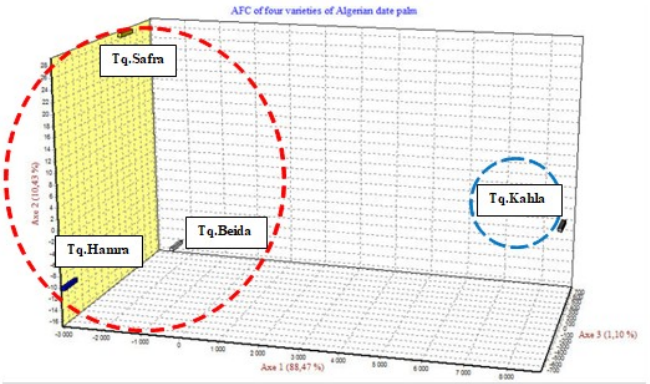


**Fig. 6.** The factorial correspondence analysis (FCA) results showing the relationship between the four cultivars of date palm.

(STW) (r = 0.63), which might explain the good environmental performances of this cultivar compared with the others. The two traits of protuberance frequency (PF) (qualitative trait) and palm length (PAL) (quantitative trait) are assembled in the cultivar Tq. hamra, which might be the reason behind the highest fruit weight due to enhanced photosynthesis mechanisms in a large area of the PAL. The average length of palms of the four cultivars was about 347.4 cm (Table 4), which is higher than the average palm length measured in the Deglet Nour cultivar (320 cm) (28) but lower than the average palm length of Touat cultivar (350.5 cm) (29).

Other quantitative characteristics, namely spines thickness (ST) (r = 0.71), spacing index (SI) (r = 0.89), spikelet numbers per bunch (SPND) (r = 0.85) and spathe length (SPL) (r = 0.85) were correlated with the Tq. beïda cultivar. This accession is particularly has well-developed palm and pinnae compared with the other accessions.

With regard to qualitative characteristics, and contrary to the outputs of principal component analysis (PCA), a multi-component analysis (MCA) sorted three major groups and nine minor groups scattered inside the main three groups (Fig. 3). The populations of the black-fruited cultivar (Tq. kahla) fall into four minor groups (groups 1, 2, 3 and 4). Group 1 was the most representative of the variety, and it was characterized by an olive-green palm, ellipsoidal fruits, black fruits and well-developed brown seeds. The two cultivars Tq. safra (yellow fruits) and Tq. beïda (white fruits) fall within group 9, characterized mainly by large ellipsoidal fruits, oblique flat bottom and fusiform seeds. The populations of the cultivar Tq. hamra fall into two groups (groups 6 and 7) characterized by distinct phenotypic morphologies, such as cylindrical elongated fruits and floury texture with good adhesion of the chalice and light-brown seeds (beige seed colour).

In general, qualitative traits of fronds and inflorescence have less distinctive effect than the qualitative traits of seeds and fruits, which provide better idiosyncratic traits to recognize and differentiate Taqerbucht date cultivars easily. Similar findings have previously reported on other cultivars (2). On the other hand, the number of alleles per primer detected in our study was relatively low compared to an earlier study (30). This might be due to the low number of cultivars being investigated. However, reports are on 221 different alleles in 6 Mauritanian date palm cultivars using 14 markers with an average of 2,57 alleles per locus (31). We, however, could not detect any allele corresponding to the three markers mPdCIR07, mPdCIR044 and mPdCIR063 (absence of PCR amplification in the DNA of the four analyzed cultivars). Similar observations have been reported previously for these markers in other cultivars (16, 31, 32).

Our result suggests high genetic diversity in four Algerian date cultivars compared to Tunisian cultivars (33). However, genetic polymorphisms seem to be less than in Sudan date cultivars (34). Our findings concord with reports on other Algerian, Moroccan and Tunisian date palms cultivars using microsatellites or isoenzymes markers (35, 36). The majority of microsatellites markers are highly polymorphic, except the mPdCIR057 marker that is monomorphic. They show significant genetic diversity and deviation from EHW, with a high heterozygosity deficiency and negative *Fis* values.

The four date cultivars showed high genetic diversity with high levels of observed heterozygosity value (*Ho*) in comparison with five SSR within 26 Tunisian cultivars (30), 14 SSR within 46 cultivars, or 16 SSR within 11 Moroccan cultivars (31). Excess heterozygosity manifested by negative *Fis* values in the four cultivars. Only the cultivar Tq kahla (black fruits) showed seven specific alleles (>50%), confirming its origin belonging to the south of Algeria. The *Fst* suggests the presence of genetic differentiation between the cultivars, which might result from geographic separation, distance, climate conditions and difficulty of exchanging vegetative materiel (30, 36), though the cultivar Tq.kahla seems to be differentiated from the three other cultivars, but there is no differentiation between the two cultivars Tq. safra and Tq. beïda (Fst= 0.000). This result confirmed by molecular variance analysis (Fig. 2), in which the total genetic diversity of Algerian date palm is strongly represented within the individual rather than among cultivars.

The phylogenetic tree constructed using Nei's minimum genetic distance among four cultivars shows two distinct groups (Fig. 3), confirmed by FCA analysis which shows two groups (Fig. 4). The first group comprises the three cultivars Tq. safra, Tq. beïda and Tq. hamra, while the second group was formed lonely by the cultivar Tq. Kahla.

## Conclusion

Morphological and genetic analyses show genetic variations within four Taqerbucht date palm cultivars (Tq. beida cultivar, Tq hamra, Tq kahla and Tq safra). Quantitative and qualitative variability analyses prove to be helpful to highlight the effectiveness of date palm descriptors, though some cultivars seemed to be mixed within other cultivars. The most important divergence criteria are the form consistency, plasticity, texture and taste of the fruit. Through intra-cultivar analysis at the interregional level of Adrar, environmental conditions have an impact in particular on the weight of the fruits and seeds, the consistency, the taste and the surface of the seed. In perspective, it would be interesting to broaden the analyses to include other cultivars from southern Algeria to characterize their resistance to bayoud disease and look for resistant genes toward breeding new disease tolerance cultivars.

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## Authors' contributions

BS carried out the Survey in palm groves Biometric analysis Molecular analysis in laboratory. AAA

participated in statistical analysis and genetic analysis. AMM participated in analysis of cultivars using SSR in laboratory. KH Survey in palm groves Biometric analysis. LA Survey in palm groves. KM participated in Survey in palm groves Biometric analysis. MA participated in Redaction correction. KL participated in Supervisor of all tests Statistical analysis Genetic analysis Redaction. All authors read and approved the final manuscript.

## Conflict of interests

Authors do not have any conflict of interests.

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