ASSESSMENT OF PHENOTYPIC DIVERSITY IN TUNISIAN CARROT (Daucus carota subsp. sativus) LANDRACES

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ABSTRACT
Knowledge of the morphological diversity of a germplasm collection is fundamental for genebank managers and plant breeders. The main objective of the present work was to characterize 33 landraces of carrot from 13 different regions of Tunisia, based on 34 agromorphological characters related to leaves and roots. The Shannon-Weaver Diversity (H') index was used to study the phenotypic diversity. The estimated H' ranged from 0.19 for core colour compared to cortex colour (RCCCC) to 0.99 for leaf division (LD). Analysis of variance revealed significant differences among landraces for all quantitative characters. Stepwise multivariate analyses were carried out to identify the useful characters that can distinguish among landraces. This study showed that qualitative characters were the best for the delimitation of landraces in this collection. Cluster analysis permitted the subdivision of carrot collection into four distinct groups independent of their geographic distribution. This information will be helpful to curators in the management and improvement of carrot germplasm in Tunisia.

Keywords: Carrot, morphological characterization, ANOVA, multivariate analyses.

1. INTRODUCTION
The genus Daucus includes about 25 recognized species world-wide. The most widespread and economically important species, Daucus carota L., occurs on almost every continent. It is found in wild or cultivated form throughout the Mediterranean, southwest Asia, Africa, Australia, New Zealand and the Americas (Peterson and Simon, 1986; Vaughan and Geissler, 2009). Central Asia is considered the center of origin of cultivated carrot, which represents a large genetic variation (Maksylewicz and Baranski, 2013; Iorizzo et al., 2013). At present, large genetic variation is observed in cultivated carrot (Daucus carota subsp. sativus (Hoffm.) Arcang.) due to the fast spread of carrot ancestors from their center of origin to distant geographical regions, and to the lack of control of random cross pollination among cultivated and wild forms. Edible carrot is one of the main sources of dietary pro-vitamin A carotenoids (Simon, 1990). Variation in the
carotenoid content and composition largely depends on the cultivar. The intensive selection on carrot led to a morphological diversity observed in leaves and roots with the first domesticates having purple and yellow roots between 11th and 15th centuries in Central Asia, Asia Minor, Western Europe and England (Banga, 1963). Orange carrot roots were domesticated in Europe between 15th and 16th centuries (Banga, 1957; Stolarczyk and Janick, 2011). Among Mediterranean regions, Tunisia is considered a center of biodiversity for Daucus and many other crops, with Tunisia having a great diversity of ecosystems and climates (Pottier Alapetite, 1979; Le Floc’het al., 2010). Carrots are widely cultivated throughout Tunisia, with the prevalence in the center (Sidi Bouzid, Kairouan and Sfax), the south (oasis regions), the coast (Nabeul, Monastir and Mahdia), and the north of the country (Kef and Seliana). Annual carrot production is 218,645 tons, representing 5% of total vegetable production. Carrot is produced on 6700 ha (~94% in the winter crop and 6% in the summer crop; DGPA, 2015). Carrot landraces are genetically heterogeneous, resulting from natural processes and farmers’ practices. However, the large genetic diversity pooled in landraces is not exploited by carrot improvement programs because of the lack of information on the agro-morphological and molecular characterization of the germplasm. Recently, Mezghaniet al. (2014, 2017) examined the morphological variation within a Daucus collection conserved at the National Gene Bank of Tunisia using fruit, vegetative and flower data. Relative to D. carota, they recognized the following subspecies: capillifolius (Gilli) Arbizu, carota (L.), gummifer (Syme) Hook. fil and sativus with high degrees of diversity. However, the large diversity regarding local germplasm for cultivated carrot needs to be studied based on agro-morphological, biochemical and molecular characterization. Thus, the aims of this study are (1) the morphological characterization of carrot landraces collected from the major growing regions of the country using several vigour descriptors related to leaf and root and (2) the analysis of genetic variation among the accessions using uni and multivariate statistical analysis of the data. This information will guide the curators in the formulation and prioritization of future conservation activities especially in the field of carrot germplasm exploration and enhancement, and guide breeders into choice of germplasm.

2. MATERIALS AND METHODS

2.1. Plant material

The study material consisted of 33 carrot landraces collected during the harvest period extending between December 2015 and February 2016 from 13 localities belonging to the main cultivation regions in Tunisia. Each accession is represented by 15 plants (roots and leaves parts) collected from the fields and seeds from the farm store. Passport data and an inventory number were assigned for each accession according to the National Gene Bank of Tunisia database and full details are available at the Germplasm Resources Information Network - GRIN (http://www.tn-grin.nat.tn/gringlobal/search.aspx). The collection and geographic position are displayed in figure 1.
Figure 1: Geographic distribution of carrot collection used in this study. The names of the provinces and locations are in bold. NGBTUN numbers are permanent identification assigned to accessions maintained at the National Gene Bank of Tunisia.

2.2. Morphological characterization

Carrot landraces were examined for 15 quantitative and 19 qualitative traits related to roots and leaves (Table 1). The selection of characters was made following the descriptors lists of IPGRI (International Plant Genetic Resources Institute, 1998) and UPOV (International Union for the Protection of New Varieties of Plants, 2007). Quantitative traits (length, width and diameter) were measured with a ruler or caliper, root weight with an electronic balance and root firmness with a penetrometer, while qualitative traits were evaluated by attributing a code to each character state.
Table 1: Morphological descriptors, descriptor states, their codes for numerical analysis, frequency distribution and diversity index of carrot landraces in Tunisia

| Trait/descriptor          | Source | Descriptor acronym | Type | Description state | Class | Frequency (%) | Diversity Index (H') |
|---------------------------|--------|--------------------|------|------------------|-------|---------------|----------------------|
| Leaf                      |        |                    |      |                  |       |               |                      |
| Crown width               | UPOV   | CW                 | QL   | Narrow           | 3     | 66.4          | 0.75                 |
|                           |        |                    |      | Medium           | 5     | 25.1          |                      |
|                           |        |                    |      | Broad            | 7     | 8.5           |                      |
| Leafnumber                | IPGRI  | LN                 | QN   | Low (≤5.2)       | 1     | 6.1           | 0.55                 |
|                           |        |                    |      | Medium (5.2-14.29) | 2   | 80.6          |                      |
|                           |        |                    |      | High (≥14.29)    | 3     | 13.3          |                      |
| Leaflength (cm)           | IPGRI  | LL                 | QN   | Short (≤42.4)    | 1     | 13.9          | 0.78                 |
|                           |        |                    |      | Intermediate (42.4-73.8) | 2   | 66.7          |                      |
|                           |        |                    |      | Elongated (≥73.8) | 3     | 19.4          |                      |
| Leafwidth (cm)            | IPGRI  | LW                 | QN   | Narrow (≤16.5)   | 1     | 13.3          | 0.69                 |
|                           |        |                    |      | Intermediate (16.5-36.1) | 2   | 73.4          |                      |
|                           |        |                    |      | Wide (≥36.1)     | 3     | 13.3          |                      |
| Leaf division             | UPOV   | LD                 | QL   | Fine             | 3     | 30.6          | 0.99                 |
|                           |        |                    |      | Medium           | 5     | 39.7          |                      |
|                           |        |                    |      | Coarse           | 7     | 29.7          |                      |
| Intensity of green colour | UPOV   | LIGC               | QL   | Light            | 3     | 33.0          | 0.91                 |
|                           |        |                    |      | Medium           | 5     | 51.2          |                      |
|                           |        |                    |      | Dark             | 7     | 15.8          |                      |
| Leafhairiness             | IPGRI  | LH                 | QL   | Sparse           | 3     | 73.0          | 0.70                 |
|                           |        |                    |      | Intermediate     | 5     | 13.7          |                      |
|                           |        |                    |      | Dense            | 7     | 13.3          |                      |
| Leafletsnumber            | IPGRI  | LIN                | QN   | Low (≤21.3)      | 1     | 9.4           | 0.59                 |
|                           |        |                    |      | Medium (21.3-28.7) | 2   | 79.4          |                      |
|                           |        |                    |      | High (≥28.7)     | 3     | 11.2          |                      |
| Length of primary basal leaflet (cm) | IPGRI  | LPBL               | QN   | Short (≤10.1)    | 1     | 15.1          | 0.76                 |
|                           |        |                    |      | Intermediate (10.1-22.6) | 2   | 68.8          |                      |
|                           |        |                    |      | Elongated (≥22.6) | 3     | 16.1          |                      |
| Number of segments of primary basal leaflet | IPGRI  | NSPBL              | QN   | Low (≤15.0)      | 1     | 17.9          | 0.88                 |
|                           |        |                    |      | Medium (15.0-19.7) | 2   | 57.3          |                      |
|                           |        |                    |      | High (≥19.7)     | 3     | 24.8          |                      |
| Foliage coverage          | IPGRI  | FC                 | QL   | Sparse           | 3     | 42.4          | 0.98                 |
|                           |        |                    |      | Dense            | 7     | 57.6          |                      |
| Petioleanthocyanincolouration | IPGRI  | PCP                | QL   | Unicoloured      | 1     | 72.8          | 0.55                 |
|                           |        |                    |      | Slightly coloured | 3     | 19.7          |                      |
|                           |        |                    |      | Intermediate     | 5     | 6.9           |                      |
|                           |        |                    |      | Strongly coloured | 7     | 0.6           |                      |
| Petiolethickness (mm)     | IPGRI  | PT                 | QN   | Narrow (≤3.6)    | 1     | 12.4          | 0.72                 |
|                           |        |                    |      | Intermediate (3.6-7.4) | 2   | 70.9          |                      |
|                           |        |                    |      | Wide (≥7.4)      | 3     | 16.7          |                      |
| Root                      |        |                    |      |                  |       |               |                      |
| Root length (cm)          | IPGRI  | RL                 | QN   | Short (≤21.4)    | 1     | 16.0          | 0.77                 |
| Description | Designation | Code | Country | Number | UPOV | QL |
|-------------|-------------|------|---------|--------|------|----|
| Root diameter at the shoulder (mm) | IPGRI | RDS | QN | Narrow (≤32.4) | 1 | 12.1 | 0.74 |
| | | | | Intermediate (32.4-53.0) | 2 | 70.0 |
| | | | | Wide (≥53.0) | 3 | 17.9 |
| Root diameter at the medium (mm) | IPGRI | RDMD | QN | Narrow (≤27.9) | 1 | 12.7 | 0.68 |
| | | | | Intermediate (27.9-45.2) | 2 | 74.3 |
| | | | | Wide (≥45.2) | 3 | 13.0 |
| Root diameter at the tip (mm) | IPGRI | RDTi | QN | Narrow (≤14.0) | 1 | 15.8 | 0.77 |
| | | | | Intermediate (14.0-31.7) | 2 | 68.2 |
| | | | | Wide (≥31.7) | 3 | 16.0 |
| Root weight (Kg) | IPGRI | RW | QN | Light (≤0.09) | 1 | 5.5 | 0.48 |
| | | | | Intermediate (0.09-0.40) | 2 | 84.2 |
| | | | | Heavy (≥0.40) | 3 | 10.3 |
| Root axis | IPGRI | RA | QL | Not straight | 1 | 30.3 | 0.88 |
| | | | | Straight | 2 | 69.7 |
| Root shape in longitudinal section | UPOV | RSLS | QL | Obovate | 2 | 0.6 | 0.71 |
| | | | | Medium obtriangular | 3 | 9.4 |
| | | | | Narrow obtriangular | 4 | 58.4 |
| | | | | Narrow obtriangular to narrow oblong | 5 | 17.9 |
| | | | | Narrow oblong | 6 | 13.7 |
| Root shoulder shape | UPOV | RSS | QL | Flat | 1 | 4.3 | 0.47 |
| | | | | Flat to rounded | 2 | 76.0 |
| | | | | Rounded | 3 | 16.4 |
| | | | | Rounded to conical | 4 | 0.3 |
| | | | | Conical | 5 | 3.0 |
| Root tip shape | UPOV | RTS | QL | Blunt | 1 | 14.9 | 0.88 |
| | | | | Slightly pointed | 2 | 30.3 |
| | | | | Strongly pointed | 3 | 54.8 |
| Root external colour | UPOV | REC | QL | Yellow | 2 | 22.1 | 0.75 |
| | | | | Orange | 3 | 57.9 |
| | | | | Pinkish red | 4 | 17.2 |
| | | | | Purple | 6 | 2.8 |
| Anthocyanin colouration of shoulder skin | UPOV | RACSS | QL | Absent | 1 | 37.6 | 0.95 |
| | | | | Present | 9 | 62.4 |
| Extent of green colour of shoulder skin | UPOV | REGCSS | QL | Absent or very small | 1 | 56.6 | 0.6 |
| | | | | Small | 3 | 1.2 |
| | | | | Medium | 5 | 2.8 |
| | | | | Large | 7 | 39.4 |
| Surface ridging | UPOV | RSR | QL | Absent or very weak | 1 | 69.1 | 0.65 |
| | | | | Weak | 3 | 27.6 |
| | | | | Medium | 5 | 3.3 |
| Core diameter (mm) | IPGRI | RCD | QN | Small (≤14.1) | 1 | 14.6 | 0.72 |
| | | | | Intermediate (14.1-25.2) | 2 | 71.5 |
| | | | | Large (≥25.2) | 3 | 13.9 |
| Cortex diameter (mm) | UPOV | RCcortD | QN | Narrow (≤2.2) | 1 | 11.3 | 0.99 |
Quantitative characters were converted to phenotypic classes with the class boundaries as described by Jaradat et al. (2004); QN: quantitative, QL: qualitative.

2.3. Statistical analyses

Data analyses were performed using statistical procedures in SAS 9.1 software (SAS 1990). Simple statistics such as means and coefficient of variation were used on quantitative parameters to compare the variation among the landraces. A variance analysis (ANOVA) was performed and then the averages were compared by Duncan’s multiple range test. A Pearson correlation analysis was then carried out to estimate the relationship between the studied variables. The following multivariate analyses were performed to evaluate the contribution of each quantitative and qualitative character to the total variation: Principal component analysis (PCA), factorial correspondence analysis (FCA) and hierarchical cluster analysis (HCA) were conducted on quantitative, qualitative and mixed data respectively. For calculating the diversity parameters, the overall entry mean value and the standard deviation were used to convert quantitative characters into qualitative ones (Jaradat et al., 2004) and frequencies were obtained from class intervals. The diversity was measured for each morphological character by using the standardized Shannon–Weaver (Shannon and Weaver, 1949; as referred by Al Khanjari et al., 2008). Diversity Index, designed as H’ had the formula: $H' = \sum p_i \log_2 p_i / \log_2 n$, where $p_i = frequency proportion of each descriptor state and n = number of states for each descriptor. The diversity index was coded as high ($H' \geq 0.60$), intermediate (0.40≤$H'<0.60$) or low (0.10≤$H'<0.40$) as described by Eticha et al. (2005).

3. RESULTS

3.1. Diversity analysis

Large natural variation was found among landraces for the majority of traits (Table 1). The diversity index ($H'$) ranged from 0.19 for core colour compared to cortex colour (RCCCC) to 0.99 for leaf division (LD) with an overall mean of 0.69. The majority of traits (13 qualitative and 11 quantitative) showed a high level of polymorphism ($H' \geq 0.6$). Intermediate variation (0.4≤$H'<0.6$) was observed in 9 characters. Core colour compared to cortex colour (RCCCC) was the only character exhibiting low level of variation ($H' = 0.19$). High variation indicates equitable distribution of the different states while low variation indicates the dominance of one character state over the others as shown by frequency distribution (Mengistu et al., 2015). Research performed by Mezghani et al. (2017), on morphological variation of 45 Daucus carota L. accessions in Tunisia showed high overall mean diversity indexes in quantitative ($H' = 0.77$) and qualitative ($H' = 0.75$) characters confirming that Tunisia is a principal major center of diversification for carrot and wild relatives in the Mediterranean region.

3.2. Phenetic analysis
3.2.1. Quantitative characters

Analysis of variance for 15 quantitative data showed high significant differences (p<0.0001) for all recorded traits among the landraces (Table 2). The coefficient of variation ranged from 13.58% (lowest) to 46.82% (highest) for number of leaflets (LIN) and root weight (RW) respectively. The high coefficients of variation (≥20%) observed for 7 characters signify a high degree of variability for effective selection of landraces. An important variability was also detected in morphological characters related to roots and leaves of yellow carrot accessions in Iran (Kasiriet al., 2013; Mehrabiet al., 2014). The degree of genetic variability within crop species is a function of the method of their domestication, the breeding system and the method by which it is maintained (Hamrick et al., 1979).

Table 2: Means comparison for quantitative traits in 33 Tunisian carrot landraces. Means in the same column followed by the same letter are not significantly different at P<0.05 according to Duncan’s multiple range test.

| Accession | LN  | LL  | LW  | LIN | LPBL | PT  | RL  | RDS | RDMd | RDTj | RW  | RCD | RCordD | RF  |
|-----------|-----|-----|-----|-----|------|-----|-----|-----|------|------|-----|-----|--------|-----|
| NGBTUN499 | 8.60 | 45.24 | 10.30 | 24.00 | 10.83 | 15.80 | 3.83 | 24.00 | 33.77 | 29.65 | 20.47 | 0.09 | 16.92 | 6.78 | 6.04 |
| NGBTUN499 | 8.60 | 48.60 | 16.10 | 22.40 | 15.50 | 17.01 | 5.23 | 23.50 | 40.39 | 33.40 | 21.33 | 0.11 | 19.78 | 6.28 | 6.37 |
| NGBTUN512 | 6.90 | 79.18 | 27.78 | 25.20 | 22.26 | 16.10 | 7.02 | 28.92 | 35.80 | 31.99 | 15.93 | 0.16 | 17.98 | 6.50 | 5.14 |
| NGBTUN514 | 11.20 | 73.03 | 39.78 | 26.90 | 25.39 | 17.80 | 7.14 | 26.42 | 39.51 | 39.52 | 18.42 | 0.28 | 23.53 | 7.49 | 6.14 |
| NGBTUN520 | 7.80 | 79.41 | 36.05 | 25.70 | 20.86 | 15.50 | 7.61 | 29.03 | 34.08 | 30.27 | 13.42 | 0.13 | 19.01 | 6.82 | 5.27 |
| NGBTUN521 | 9.50 | 70.50 | 32.95 | 26.00 | 22.87 | 15.40 | 7.41 | 27.14 | 58.78 | 36.72 | 19.14 | 0.21 | 20.20 | 6.47 | 5.98 |
| NGBTUN522 | 7.00 | 43.23 | 26.50 | 72.40 | 26.69 | 18.70 | 7.54 | 29.32 | 42.64 | 36.08 | 16.39 | 0.24 | 21.29 | 6.97 | 6.42 |
| NGBTUN523 | 10.00 | 71.91 | 33.20 | 28.50 | 25.26 | 18.20 | 8.05 | 25.00 | 42.02 | 35.45 | 18.67 | 0.26 | 21.99 | 7.49 | 3.25 |
| NGBTUN524 | 7.90 | 85.44 | 42.42 | 27.60 | 27.71 | 17.00 | 9.30 | 26.90 | 40.50 | 34.77 | 20.67 | 0.26 | 22.49 | 6.81 | 4.39 |
| NGBTUN525 | 9.00 | 82.69 | 46.37 | 29.80 | 25.41 | 17.50 | 9.46 | 31.10 | 42.03 | 36.63 | 21.39 | 0.30 | 21.66 | 7.34 | 5.05 |
| NGBTUN527 | 17.40 | 52.10 | 33.80 | 26.60 | 16.02 | 18.20 | 4.84 | 24.40 | 44.66 | 34.40 | 26.73 | 0.20 | 18.82 | 7.70 | 5.49 |
| NGBTUN528 | 15.10 | 40.75 | 23.90 | 26.20 | 11.25 | 18.60 | 3.89 | 27.70 | 43.37 | 36.01 | 24.63 | 0.20 | 17.34 | 8.37 | 4.64 |
| NGBTUN529 | 16.00 | 35.05 | 28.30 | 24.60 | 10.79 | 17.00 | 3.93 | 25.90 | 42.75 | 37.21 | 27.37 | 0.22 | 21.04 | 8.79 | 5.36 |
| NGBTUN530 | 11.30 | 55.19 | 28.40 | 24.20 | 17.32 | 17.60 | 4.54 | 28.55 | 55.39 | 42.94 | 30.85 | 0.43 | 25.18 | 9.78 | 5.57 |
| NGBTUN531 | 11.60 | 43.35 | 27.50 | 25.00 | 14.59 | 18.40 | 4.34 | 29.05 | 49.36 | 41.89 | 29.72 | 0.29 | 21.57 | 8.92 | 5.12 |
| NGBTUN532 | 9.50 | 54.49 | 25.20 | 22.40 | 13.57 | 17.20 | 4.78 | 26.20 | 45.24 | 39.41 | 18.80 | 0.20 | 21.23 | 9.50 | 4.70 |
| NGBTUN534 | 14.70 | 51.25 | 24.70 | 26.90 | 15.09 | 18.20 | 5.06 | 23.25 | 51.14 | 38.41 | 18.62 | 0.26 | 19.42 | 10.21 | 4.99 |
| NGBTUN537 | 15.20 | 41.26 | 26.10 | 23.80 | 12.81 | 17.40 | 4.72 | 29.57 | 42.90 | 37.61 | 12.98 | 0.28 | 27.92 | 11.74 | 4.92 |
| NGBTUN539 | 13.70 | 40.00 | 25.50 | 23.80 | 14.92 | 16.40 | 5.17 | 30.45 | 36.24 | 26.37 | 10.65 | 0.14 | 15.81 | 5.82 | 3.68 |
| NGBTUN540 | 13.80 | 60.65 | 34.80 | 26.00 | 19.30 | 20.60 | 5.97 | 26.94 | 71.32 | 60.91 | 39.02 | 0.62 | 26.14 | 15.74 | 5.59 |
| NGBTUN541 | 10.00 | 41.20 | 20.30 | 22.00 | 11.60 | 15.20 | 4.87 | 24.75 | 37.05 | 30.13 | 16.98 | 0.12 | 14.52 | 5.83 | 4.65 |
| NGBTUN547 | 6.90 | 54.60 | 22.00 | 24.00 | 12.45 | 18.00 | 4.31 | 17.72 | 59.26 | 51.32 | 34.25 | 0.25 | 24.27 | 12.47 | 6.03 |

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Table 3: Pearson correlation coefficients among 15 quantitative traits of 33 Tunisian horticultural characteristics is of primary importance in the field of crop improvement. Linkage relationships can be used to increase breeding efficiency by allowing earlier selection and reducing plant population size during selection (Nasrabadi et al., 2012).

| Trait       | LW | LL | LW | LLN | LPBL | NSPBL | PT | RL  | RDS | RDMd | RDTi | RW  | RCD | RCoord |
|-------------|----|----|----|-----|------|-------|----|-----|-----|------|------|-----|-----|--------|
| **NGBTUN556** | 0.64 | 0.52 | 0.59 | 0.78 | 0.79 | 0.77 | 0.63 | 0.69 | 0.74 | 0.68 | 0.55 | 0.52 | 0.37 | 0.25 |
| | | | | | | | | | | | | | | |
| **NGBTUN558** | 0.69 | 0.55 | 0.76 | 0.83 | 0.84 | 0.81 | 0.74 | 0.79 | 0.77 | 0.72 | 0.62 | 0.57 | 0.44 | 0.36 |
| | | | | | | | | | | | | | | |
| **NGBTUN559** | 0.89 | 0.64 | 0.75 | 0.77 | 0.77 | 0.79 | 0.76 | 0.80 | 0.74 | 0.69 | 0.60 | 0.52 | 0.40 | 0.33 |
| | | | | | | | | | | | | | | |
| **NGBTUN560** | 6.00 | 1.45 | 0.26 | 0.30 | 0.15 | 0.16 | 0.20 | 0.20 | 0.15 | 0.14 | 0.11 | 0.10 | 0.07 | 0.07 |
| | | | | | | | | | | | | | | |
| **NGBTUN561** | 9.60 | 0.54 | 0.37 | 0.33 | 0.29 | 0.29 | 0.26 | 0.26 | 0.23 | 0.21 | 0.18 | 0.17 | 0.12 | 0.10 |
| | | | | | | | | | | | | | | |
| **NGBTUN562** | 7.70 | 0.55 | 0.55 | 0.59 | 0.57 | 0.60 | 0.57 | 0.60 | 0.58 | 0.55 | 0.50 | 0.46 | 0.39 | 0.32 |
| | | | | | | | | | | | | | | |
| **NGBTUN563** | 6.70 | 0.56 | 0.25 | 0.20 | 0.13 | 0.14 | 0.14 | 0.14 | 0.13 | 0.13 | 0.10 | 0.10 | 0.08 | 0.08 |
| | | | | | | | | | | | | | | |
| **NGBTUN564** | 7.70 | 0.59 | 0.52 | 0.56 | 0.57 | 0.60 | 0.59 | 0.62 | 0.60 | 0.56 | 0.50 | 0.49 | 0.42 | 0.36 |
| | | | | | | | | | | | | | | |
| **NGBTUN565** | 7.50 | 0.59 | 0.50 | 0.56 | 0.57 | 0.60 | 0.59 | 0.62 | 0.60 | 0.56 | 0.51 | 0.49 | 0.44 | 0.38 |
| | | | | | | | | | | | | | | |
| **NGBTUN566** | 7.70 | 0.59 | 0.50 | 0.56 | 0.57 | 0.60 | 0.59 | 0.62 | 0.60 | 0.56 | 0.51 | 0.49 | 0.44 | 0.38 |
| | | | | | | | | | | | | | | |
| **NGBTUN567** | 6.70 | 0.56 | 0.25 | 0.20 | 0.13 | 0.14 | 0.14 | 0.14 | 0.13 | 0.13 | 0.10 | 0.10 | 0.08 | 0.08 |
| | | | | | | | | | | | | | | |
| **NGBTUN571** | 7.40 | 0.59 | 0.10 | 0.18 | 0.10 | 0.12 | 0.13 | 0.13 | 0.12 | 0.12 | 0.10 | 0.10 | 0.08 | 0.08 |
| | | | | | | | | | | | | | | |
| **NGBTUN572** | 4.00 | 0.49 | 0.20 | 0.12 | 0.09 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| | | | | | | | | | | | | | | |

** Significant at 0.1%, * significant at 5%.
Because the quantitative characters are interrelated, we conducted a principal component analysis to determine their impact. The first three principal components accounted for 71.71% of the variance (Table 4). The first principal component with an eigenvalue of 4.88 explained 32.57% of the total variability and was mainly associated with root diameter at shoulder (RDS), root diameter at the medium (RDMd) and core diameter (RCD). Principal component 2 with an eigenvalue of 4.39 accounted for 29.28% of the morphological variability and was strongly correlated with petiole thickness (PT), length of primary basal leaflet (LPBL) and leaf length (LL). Principal component 3 with an eigenvalue of 1.47 accounted for 9.85% of the total variability and was positively correlated with leaf number (LN) and root firmness (RF) but negatively correlated with root diameter at the tip (RDTi) and root weight (RW). The PCA scatterplot defined by the two principal components 1 and 2 (Figure 2) separated carrot landraces into 3 groups. The first group (G1) included accessions NGBTUN512, 514, 520, 521, 522, 523, 524 and 525 from Monastir (Moknine and Teboulba locations). The second group G2 is formed by the remaining accessions except for accession NGBTUN540 from Sfax (Hezag) which diverges from all the other accessions and formed the group G3. This accession consistently showed highest values for six quantitative traits (Table 2). The principal component analysis permitted the subdivision of the accessions independently from their geographic zones and their bioclimatic conditions. The quantitative traits may be modified variously by the environmental conditions and are usually governed by many factors or genes each contributing such a small amount of phenotype such that their individual effects cannot be detected by Mendelian methods. They do not show clear differences between individuals and form a spectrum of phenotypes which blend imperceptivity from one type to another as continuous variation (Hill, 2010).

**Table 4: Values of the first three components of PCA based on morphological quantitative characters of Tunisian carrot landraces.**

| Principal component | Axis 1 | Axis 2 | Axis 3 |
|---------------------|--------|--------|--------|
| **Eigenvalue**      | 4.88   | 4.39   | 1.47   |
| **Percentage (%)**  | 32.57  | 29.28  | 9.85   |
| **Cumulative percentage** | 32.57  | 61.85  | 71.71  |

| Character | **Eigenvalue** |
|-----------|----------------|
| LN        | 0.16 -0.44     |
| LL        | 0.05 0.40      |
| LW        | 0.19 0.37      |
| LIN       | 0.14 0.34      |
| LPBL      | 0.12 0.43      |
| NSPBL     | 0.28 0.02      |
| PT        | 0.07 0.44      |
RL   -0.04   0.22   0.12
RDS  0.40   -0.13  -0.01
RDMd 0.41   -0.10  -0.08
RDTi 0.27   0.23  -0.31
RW   0.30   -0.03  -0.31
RCD  0.35   0.08   0.19
RCortD 0.33  -0.19   0.01
RF   0.17  -0.43   0.39

Figure 2: Scatter plot grouping of 33 Tunisian carrot landraces based on the first two principal components of PCA.

3.2.2. Qualitative characters

A factorial analysis of correspondence (FAC) was carried out to detect associations and oppositions existing between carrot landraces and qualitative traits, measuring their contribution to the total variability for each factor. Table 5 shows the eigenvalue and cumulative percentage
of qualitative traits of the first three factors. Factor 1 accounted 22.59% of the total variance and was positively correlated with root external colour (REC), extent of green colour of shoulder skin (REGCSS), root branching (RB) and anthocyanin colouration of shoulder skin (RACSS). Factor 2 explained 17.52% of the total variance and was positively correlated with foliage coverage (FC), root shoulder shape (RSS), protrusion above soil (RPAS) and core colour compared to root colour (RCCCC). The scatter plot of factorial correspondence analysis defined by the first two factors (Figure 3) divided carrot accessions on the basis of the qualitative characteristics into four distinct groups. The first group (G1) included accessions NGBTUN547 and 556 from Gabes, NGBTUN564, 565, 566, 567 from Nabeul (Slimane location); NGBTUN571 and 572 from Siliana characterized as having a narrow crown width; leaves with a strong anthocyanin petiole colouration, a fine division and a medium intensity of green colour; and roots with orange skin and core colour, a small extent of green colour of shoulder skin having a rounded shape. Accessions from Gabes are characterized by a blunt root tip and a medium obtriangular root shape in longitudinal section. Whereas accessions from Siliana and Slimane exhibited a slightly pointed root tip and a narrow oblong root shape in longitudinal section. The second group (G2) formed by accessions NGBTUN558, 559, 560, 563 from Nabeul (Menzel Temime) and NGBTUN490, 499 from Nabeul (Korba) presented leaves with coarse division and medium hairiness but without anthocyanin petiole colouration. Roots are bent and have a yellow external colour, a very weak surface ridging, and a large extent of green colour of shoulder skin which is characterized by a conical shape. The third group (G3) comprised accessions NGBTUN527, 528, 529, 530, 531 from Sidi Bouzid, NGBTUN532, 534, 537, 539 from Kairouan and NGBTUN540, 541 from Sfax presented leaves with a medium division, a strongly hairiness, and a slightly to intermediate coloured petiole. Roots are pinkish red in external colour with a narrow obtriangular to narrow oblong shape in longitudinal section, a weak surface ridging, a flat shoulder shape with a very small extent of skin green colour. The fourth group (G4) formed by NGBTUN512, 514, 520, 521, 522, 523, 524 and 525 from Monastir showed an intermediate to a wide foliage width and intensely dark green leaves. Roots are yellow to orange with a strongly pointed tip shape, a white to yellow core colour, a large extent of green colour of shoulder skin and a sparse to intermediate branching. Among these accessions, there are roots with purplecolour externally. Accessions in the first group are assembled independently of their geographic origin; this could be explained by the allogamous mating system of the species or the frequent seed exchange among farmers and regional markets (Mezghaniet al., 2014). However, accessions of the second, the third and the fifth group are from the same geographic zone, this could be explained by a local human selection or a suitable adaptation of accessions to their specific habitat conditions.
Table 5: Values of the first three factors of FCA based on morphological qualitative characters of Tunisian carrot landraces.

| Principal factor | Factor 1 | Factor 2 | Factor 3 |
|------------------|----------|----------|----------|
| Eigenvalue       | 0.44     | 0.38     | 0.31     |
| Percentage (%)   | 22.59    | 17.52    | 11.29    |
| Cumulative percent | 22.59 | 40.12  | 51.4     |

| Character | Eigenvalue |
|-----------|------------|
| RA        | 0.56       |
| RSLS      | 0.32       |
| RSS       | 0.64       |
| RTS       | 0.28       |
| REC       | 1.18       |
| RB        | 1.10       |
| RACSS     | 1.10       |
| REGCSS    | 1.12       |
| RSR       | 0.33       |
| RCC       | 0.59       |
| RCCCC     | -0.46      |
| RFCDTS    | 0.10       |
| FC        | -0.01      |
| RPAS      | 0.21       |
| CW        | 0.40       |
| LD        | 0.16       |
| LIGC      | 0.69       |
| PCP       | 0.34       |
| LH        | 0.20       |
| FW        | -0.21      |
Figure 3: Scatter plot grouping of 33 Tunisian carrot landraces based on the first two principal factors of FCA.

3.3. Grouping of landraces using quantitative and qualitative characters

A dendrogram (Figure 4) combining quantitative and qualitative characters was carried out to evaluate the general pattern of variance and to establish relationship among carrot landraces. At an average distance of 1.0, hierarchical clustering defines two major clusters including the same groups (G1 to G4) identified by FCA. Landraces of G2 from Nabeul (Menzel Temime and Korba) and G4 from Monastir (Moknine and Teboulba) fell together in cluster Cl2 whereas cluster Cl1 included landraces from Sidi Bouzid, Kairouan, Sfax (G3) and Gabes, Slimane (Nabeul) and Siliana (G1). This hierarchical classification provided evidence that landraces are clustered independently to their geographic origins. Abdellaouiet al. (2010) and Lahbib et al. (2013) reported that the cluster pattern of barley and pepper landraces in Tunisia is not always related to geographical distribution. Diversity detected within accessions could mainly be attributed to diverse agro-climatic conditions in Tunisia. The intraregional and interregional diversity may be as a valuable source for crop improvement (Lahbib et al., 2012).
Figure 4: Dendrogram obtained from cluster analysis of 33 Tunisian carrot landraces using the UPGMA.
4. CONCLUSION

The present study uses morphological characterization of 33 Tunisian carrot landraces collected from diverse regions of Tunisia to evaluate quantitative and qualitative parameters related to roots and leaves. We here document a high morphological variability within landraces. These results, in combination with previous ones (Mezghaniet al., 2014, 2017) confirm that Tunisia is a principal major center of diversification for Daucus in the Mediterranean region. This information will be helpful to curators in the management and improvement of carrot germplasm in Tunisia and worldwide.

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