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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 agro-morphological 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 (Maksylewiczand Baranski, 2013; Iorizzoet 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

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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; Stolarczykand 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.

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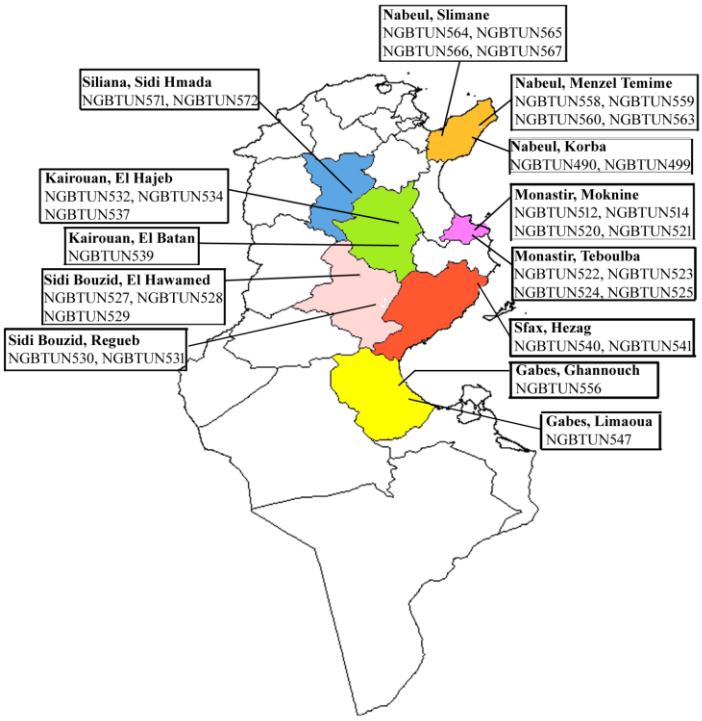

Figure 1: Geographic distribution of carrot collection used in this study. The names of the provinces and locations are in bold. NGBTUN numbers are permanent identification assigned to accessions maintained at the National Gene Bank of Tunisia.

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, whilequalitative traits were evaluated by attributing a code to each character state.

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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*	*Descriptor state* [a]	*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	LlN	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	Uncoloured	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

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				Intermediate (21.4-31.8)	2	68.2	
				Elongated (≥31.8)	3	15.8	
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	RCortD	QN	Narrow (≤5.2)	1	11.5	0.59

				Intermediate (5.2-10.5) Wide (≥10.5)	2 3	79.1 9.4	
Core colour	UPOV	RCC	QL	White Yellow Orange Pinkish red	1 2 3 4	2.5 73.0 22.4 2.1	0.53
Core colour compared to root cortex colour	UPOV	RCCCC	QL	Lighter Same Darker	1 2 3	94.9 4.8 0.3	0.19
Root branching	IPGRI	RB	QL	Absent Sparse Intermediate	0 3 5	85.1 13.0 1.9	0.43
Flesh colour distribution in transverse section	IPGRI	RFCDTS	QL	Colour in two distinct outer and inner cores Colour radially distributed in stellate pattern Colour radially distributed from inner core	2 3 4	79.1 20.6 0.3	0.48
Root firmness	UPOV	RF	QN	Low (≤3.3) Intermediate (3.3-5.9) High(≥5.9)	1 2 3	16.4 67.9 15.7	0.77
Protrusion above soil	UPOV	RPAS	QL	Small Medium Large	3 5 7	6.0 36.4 57.6	0.77

aQuantitative characters were converted to phenotypic classes with the class boundaries as described by Jaradatet 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 (Jaradatet 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 Khanjariet al., 2008).Diversity Index, designed as H' has the formula: $H' = -\sum pi (\log2\ pi) / \log2\ n$, where pi = 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 ($0.40 \leq H' < 0.60$) or low ($0.10 \leq H' < 0.40$) as described by Etichaet 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 ($0.4 \leq H' < 0.6$) 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 Mezghaniet 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

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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 (LlN) 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 significant different at P<0.05 according to Duncan's multiple range test.

Accession	*LN*	*LL*	*LW*	*LlN*	*LPBL*	*NSPBL*	*PT*	*RL*	*RDS*	*RDMd*	*RDTi*	*RW*	*RCD*	*RCortD*	*RF*
NGBTUN490	8.60fgh	45.24klm	10.30q	24.00fgh	10.83mn	15.80fgh	3.83jk	24.00ijk	33.77mno	29.65lmn	20.47jkl	0.09j	16.92lmn	6.78klm	6.04abc
NGBTUN499	8.60fgh	48.60klm	16.10nop	22.40gh	15.50hij	17.00fgh	5.23fgh	23.50ijk	40.39klm	33.40jkl	21.33jkl	0.11ij	19.78ijk	6.28klm	6.37a
NGBTUN512	6.90hi	79.18ab	27.73hij	25.20fgh	22.26bcd	16.10fgh	7.02d	28.92def	35.80mno	31.39lmn	15.93lmn	0.16hij	17.98klm	6.56klm	5.14def
NGBTUN514	11.20edf	73.03bc	39.70bc	26.80cde	25.39ab	17.80def	7.31d	26.42hij	39.51lmn	39.52efg	18.42klm	0.28cde	23.53cde	7.49ijk	6.14ab
NGBTUN520	7.80fgh	79.41ab	36.05cd	25.70fgh	20.86cd	15.50fgh	7.61cd	29.03def	34.08mno	30.27lmn	13.42mn	0.13hij	19.01klm	4.82m	5.27def
NGBTUN521	9.50fgh	70.50cd	32.95efg	26.00efg	22.87bc	15.40gh	7.61cd	27.14ghi	38.76lmn	36.72ghi	19.14klm	0.21hij	20.20ijk	6.47klm	5.59bcd
NGBTUN522	7.00hi	83.23a	26.50ijk	27.40bcd	26.69a	18.70abc	7.51d	29.32cde	42.64ghi	36.08hij	16.39lmn	0.24fgh	22.19efg	6.97klm	4.62efg
NGBTUN523	10.00fgh	71.91cd	33.20efg	28.50ab	25.28ab	18.20bcd	8.50bc	25.00hij	42.02hij	35.45ijk	18.67klm	0.26efg	21.99efg	7.49ijk	3.25ij
NGBTUN524	7.90fgh	85.44a	42.40ab	27.60abc	27.71a	17.60efg	9.30ab	26.90hij	40.50klm	34.77jkl	20.61jkl	0.26def	22.49def	6.81klm	4.39fgh
NGBTUN525	9.90fgh	82.69a	46.37a	29.80a	25.41ab	17.50fgh	9.46a	31.10ab	42.03hij	36.63ghi	21.39jkl	0.30bc	21.66fgh	7.34jkl	5.05def
NGBTUN527	17.40a	52.10hij	33.80def	26.60def	16.02fgh	18.20bcd	4.48hij	24.40hij	44.66efg	34.40jkl	26.73efg	0.20hij	18.82klm	7.70ijk	5.49bcd
NGBTUN528	15.10ab	40.75mn	23.90jkl	26.20def	11.25lmn	18.60abc	3.89jk	27.70ghi	43.37fgh	36.01hij	24.63ghi	0.20hij	17.34lmn	8.37hij	4.64efg
NGBTUN529	16.00ab	35.05n	28.30hij	24.60fgh	10.79mn	17.00fgh	3.93jk	25.90hij	42.75ghi	37.21ghi	27.37efg	0.22ghi	21.04hij	8.79ghi	5.36cde
NGBTUN530	11.10efg	55.19ghi	28.40ghi	24.20fgh	17.32ef	17.60efg	4.54hij	28.55efg	55.39bc	42.94cd	30.85bcd	0.41b	25.51abc	9.78def	5.57bcd
NGBTUN531	11.60cde	43.35lmn	27.50hij	25.00fgh	14.59klm	18.40bcd	4.34ijk	29.05def	49.36de	41.89cde	29.72cde	0.29cd	21.37ghi	8.92fgh	5.12def
NGBTUN532	9.50fgh	54.49ghi	25.20jkl	22.40gh	13.57lmn	17.20fgh	4.73hij	26.20hij	45.24efg	39.41efg	18.80klm	0.20hij	21.23ghi	9.58efg	4.70efg
NGBTUN534	14.70abc	51.25ijk	24.70jkl	26.80cde	15.09ijk	18.20bcd	5.06ghi	23.25ijk	51.14cd	38.41fgh	18.62klm	0.26efg	19.42jkl	10.21cde	4.99def
NGBTUN537	15.20ab	41.26lmn	26.10jkl	23.80fgh	12.81lmn	17.80def	4.72hji	29.57bcd	42.90ghi	37.61ghi	12.98mn	0.28cde	27.92a	11.74bc	4.92def
NGBTUN539	13.70bcd	40.60mn	25.50jkl	23.80fgh	14.92jkl	16.40fgh	5.17ghi	30.43abc	36.24lmn	26.37n	10.65n	0.14hij	15.81lmn	5.82klm	3.68hij
NGBTUN540	13.80bcd	60.65egf	34.80cde	26.00efg	19.30de	20.60a	5.97e	26.94hij	71.32a	60.91a	39.02a	0.62a	26.14ab	15.74a	5.59bcd
NGBTUN541	10.00fgh	41.20lmn	20.30mno	22.00h	11.60lmn	15.20h	4.87hij	24.75hij	37.05lmn	30.13lmn	16.98lmn	0.12hij	14.52lmn	5.83klm	4.65efg
NGBTUN547	6.90hi	54.60ghi	22.00lmn	24.00fgh	12.45lmn	18.00cde	4.31ijk	17.72l	59.26b	51.32b	34.25ab	0.25efg	24.27bcd	12.47b	6.03abc

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NGBTUN556	6.40hi	52.35hij	14.70opq	23.80fgh	13.30lmn	15.40gh	4.77hij	19.96kl	55.74bc	46.54bc	32.33bc	0.23ghi	21.86efg	10.73bcd	5.10def
NGBTUN558	6.90hi	55.76ghi	24.60jkl	24.60fgh	14.03klm	17.40fgh	5.26fgh	24.87hij	36.62lmn	35.84hij	22.08jkl	0.16hij	16.68lmn	6.82klm	2.97ij
NGBTUN559	8.90fgh	64.35ed	29.00fgh	23.00fgh	15.67ghi	17.20fgh	5.66efg	28.04fgh	44.93efg	35.93hij	22.68jkl	0.24fgh	18.09klm	7.20klm	3.14ij
NGBTUN560	7.00hi	61.45ef	26.20jkl	25.20fgh	17.15efg	19.60ab	5.89ef	27.63ghi	34.90mno	32.38klm	23.98hij	0.21hij	19.39jkl	6.33klm	2.85j
NGBTUN563	9.60fgh	54.37ghi	23.10klm	24.50fgh	14.19klm	18,60abc	5.28fgh	27.39ghi	41.21ijk	35.58ijk	28.16def	0.23ghi	19.89ijk	7.89ijk	3.26ij
NGBTUN564	7.70fgh	55.55ghi	23.50klm	25.60fgh	14.60klm	18.20bcd	5.31fgh	32.20a	39.54lmn	33.03jkl	26.14fgh	0.19hij	15.55lmn	6.91klm	3.34ij
NGBTUN565	7.50ghi	59.80fgh	25.60jkl	25.00fgh	13.77klm	18.40bcd	5.36fgh	29.79abc	40.82jkl	35.22ijk	24.37ghi	0.31bc	16.16lmn	7.40jkl	4.70efg
NGBTUN566	7.70fgh	59.80fgh	21.80lmn	23.40fgh	13.60lmn	17.40fgh	4.71hij	25.10hij	47.03def	40.50def	27.35efg	0.28cde	16.89lmn	8.82ghi	4.32fgh
NGBTUN567	6.70hi	56.25ghi	20.00mno	23.40fgh	11.95lmn	15.60fgh	4.11ijk	32.10a	39.46lmn	36.99ghi	28.05def	0.24fgh	14.37mn	7.70ijk	4.98def
NGBTUN571	7.40hi	59.10fgh	18.00mno	24.40fgh	11.00lmn	16.40fgh	3.33k	23.15ijk	33.02no	28.84lmn	21.02jkl	0.17hij	18.39klm	5.03lm	2.87j
NGBTUN572	4.00i	49.50jkl	12.10pq	25.00fgh	9.75n	17.20fgh	3.37k	22.40jk	29.48o	26.58mn	23.19ijk	0.54a	13.02n	7.18klm	3.88ghi
CV(%)	34.44	14.74	22.83	13.58	22.65	12.27	18.63	16.03	14.83	15.66	29.11	46.82	23.14	27.64	19.37
F value	9.47	25.30	17.98	2.72	19.65	3.52	25.01	5.94	17.70	14.14	9.03	8.73	5.97	10.34	12.62

Relations between quantitative traits were expressed in the correlation matrix (Table 3). According to this table, 36 morphological features were significantly correlated at the 0.05 or 0.001 significance levels. The main positive correlation appeared as follows: length of primary basal leaflet (LPBL) with leaf length (LL; r=0.86) and leaf width (LW; r=0. 77); petiole thickness (PT) with leaf length (LL; r=0.84), leaf width (LW; r=0.77) and length of primary basal leaflet (LPBL; r=0.94); root diameter at the shoulder (RDS) with root diameter at the medium (RDMd; r=0.94) and cortex diameter (RCortD, r=0.88); root diameter at the medium (RDMd) with cortex diameter (RCortD, r= 0.89) . Root weight (RW) was positively and significantly correlated with all root diameters (RDS, RDMd and RDTi) with Pearson coefficients of 0.51 or 0.53. Positive correlation between root weight and root diameter (r=0.84) was also observed in Iranian yellow carrot accessions (Kasiriet al., 2013). Information about the correlation and linkage among different 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 (Nasrabadiet al., 2012).

Table 3:Pearson correlation coefficients among 15 quantitative traits of 33 Tunisian carrot landraces.

	LN	*LL*	*LW*	*LlN*	*LPBL*	*NSPBL*	*PT*	*RL*	*RDS*	*RDMd*	*RDTi*	*RW*	*RCD*	*RCortD*
LL	-0.41*													
LW	0.32	0.61**												
LlN	0.16	0.57**	0.67**											
LPBL	-0.06	**0.86****	**0.77****	0.69**										
NSPBL	0.28	0.04	0.28	0.37	0.16									
PT	-0.12	**0.84****	**0.77****	0.66**	**0.94****	0.07								
RL	0.12	0.23	0.41*	0.21	0.29	0.11	0.32							
RDS	0.32	-0.08	0.15	0.01	0.01	0.44*	-0.06	-0.21						
RDMd	0.19	0.01	0.20	0.02	0.08	0.43*	-0.00	-0.21	**0.94****					
RDTi	-0.01	-0.2	-0.12	-0.10	-0.27	0.39*	-0.34	-0.26	0.68*	0.71**				
RW	0.04	0.08	0.18	0.22	0.08	0.51*	0.01	0.01	0.51*	0.53**	0.51*			
RCD	0.34	0.19	0.45	0.26	0.41*	0.38*	0.30	-0.08	0.61**	0.65**	0.19	0.32		
RCortD	0.34	-0.24	0.02	-0.04	-0.14	0.47*	-0.20	-0.24	**0.88****	**0.89****	0.61*	0.61**	0.62**	
RF	0.26	-0.04	0.10	0.00	0.10	-0.16	0.01	-0.17	0.36*	0.39*	0.15	0.06	0.37*	0.34*

** Significant at 0.1%,* significant at 5%.

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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 (Figure2) separated carrot landraces into 3 groups. The first group (G1) included accessions NGBTUN512, 514, 520, 521, 522, 523, 524 and 525from Monastir (Moknine and Teboulba locations). The second group G2 is formed by the remaining accessions expect 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	0.65
LL	0.05	0.40	-0.29
LW	0.19	0.37	0.17
LlN	0.14	0.34	-0.01
LPBL	0.12	0.43	-0.02
NSPBL	0.28	0.02	-0.15
PT	0.07	0.44	-0.04

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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

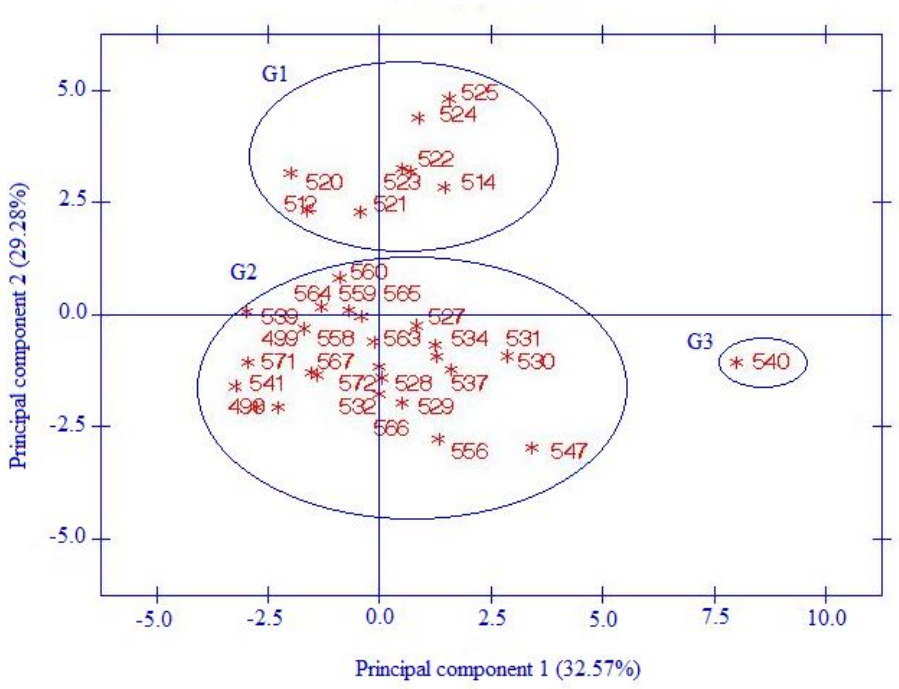

Figure 2:Scatter plot grouping of 33 Tunisian carrot landraces based on the first two principal components of PCA.

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

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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 (Figure3) 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 havinga 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 havea yellow external colour, a very weak surface ridging, and a large extent of green colour of shoulder skinwhich 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 extentof green colourof shoulder skin and a sparse to intermediate branching. Among these accessions, there are roots with purplecolourexternally. 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 firth group are from the same geographic zone, this could be explain by a local human selection or a suitable adaptation of accessions to their specific habitat conditions.

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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	0.37	
RSLS	0.32	-0.11	
RSS	0.64	1.10	
RTS	0.28	-0.22	
REC	1.18	0.50	
RB	1.10	-0.70	
RACSS	1.10	-0.01	
REGCSS	1.12	0.04	
RSR	0.33	0.16	
RCC	0.59	-0.21	
RCCCC	-0.46	1.09	
RFCDTS	0.10	0.01	
FC	-0.01	1.12	
RPAS	0.21	1.09	
CW	0.40	-0.55	
LD	0.16	0.05	
LIGC	0.69	-0.39	
PCP	0.34	0.13	
LH	0.20	0.18	
FW	-0.21	0.30	

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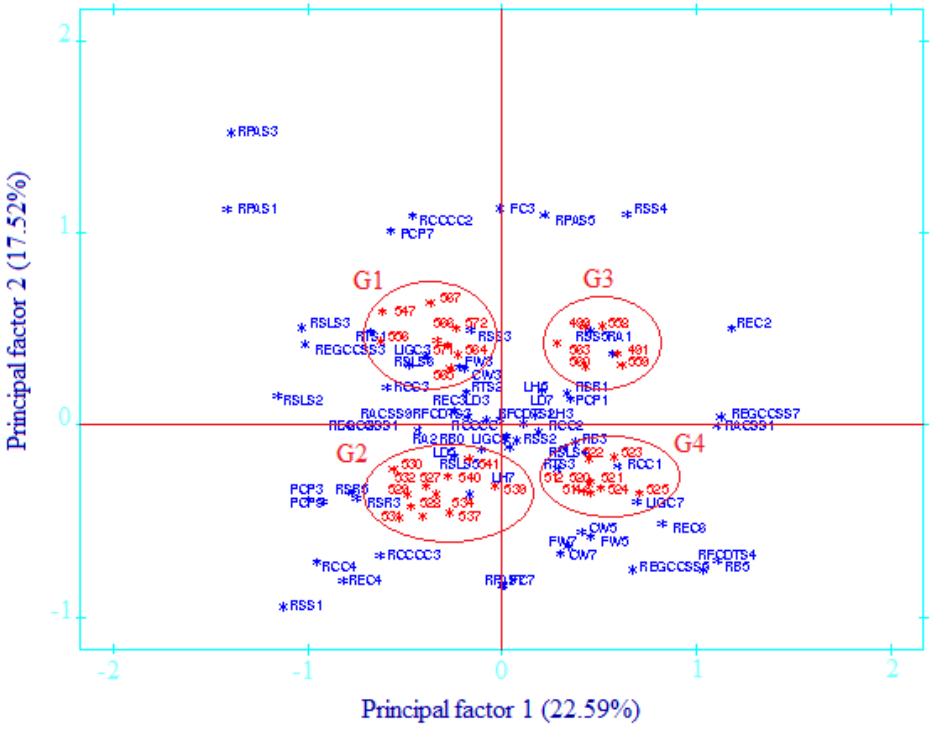

Figure 3: Scatter plot grouping of 33 Tunisian carrot landraces based on the first two principal factors of FCA.

3.3. Grouping of landraces using quantitative and qualitative characters

A dendrogram (Figure4) 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 (Moknineand Teboulba) fell together in cluster Cl1 whereas cluster Cl2 included landraces from Sidi Bouzid, Kairouan, Sfax (G3) and Gabes, Slimane (Nabeul) andSiliana (G1). This hierarchical classification provided evidence that landraces are clustered independently to their geographic origins. Abdellaouiet al. (2010) and Lahbibet 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 (Lahbibet al., 2012).

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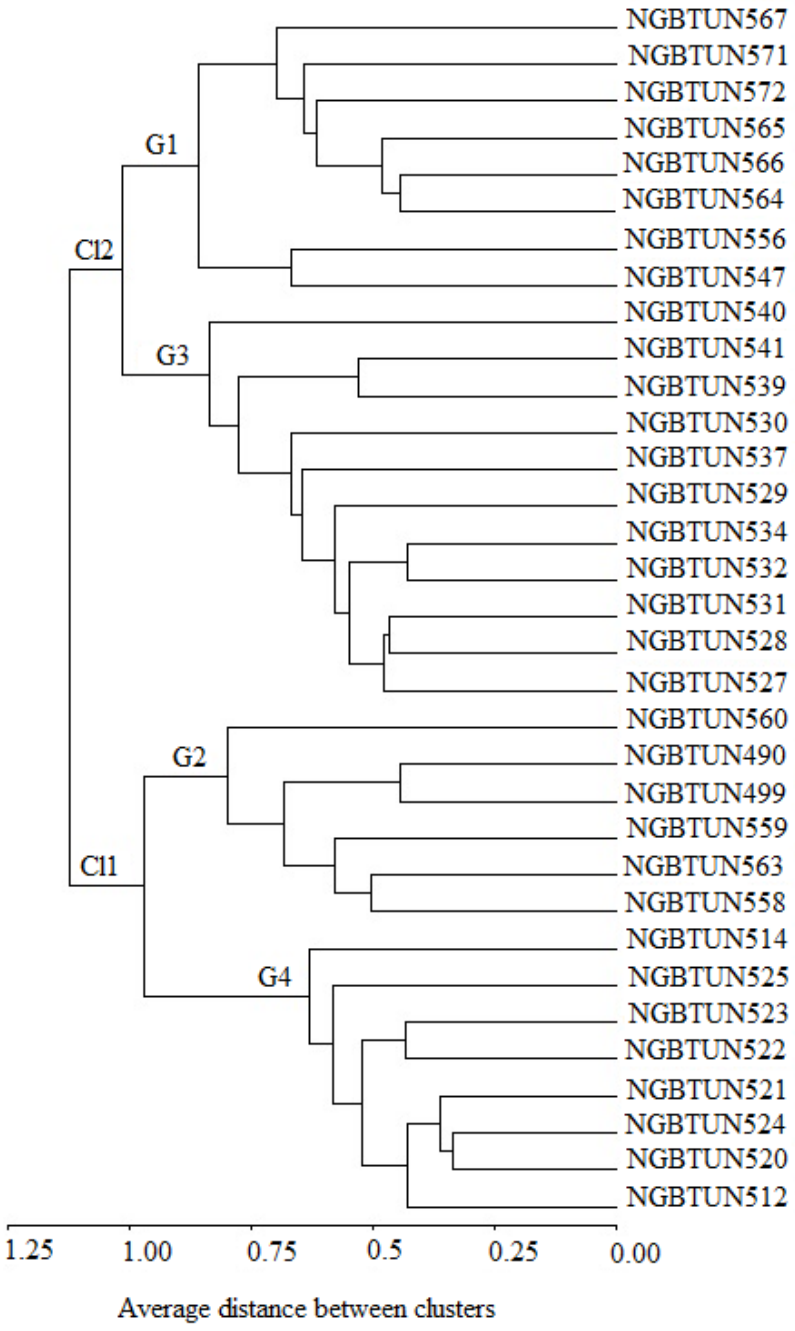

Figure 4: Dendrogram obtained from cluster analysis of 33 Tunisian carrot landraces using the UPGMA.

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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 centerof 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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