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Value adding search among a selection of Tunisian fennel (*Foeniculum vulgare* Mill.) cultivars: Diversity assessment and selection among a local fennel germplasm

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Abstract

Fennel (*Foeniculum vulgare* Mill.) is a very popular, aromatic, herbaceous plant that belongs to the Apiaceae family. It is among most important spices and medicinal species over the world. Nevertheless, in Tunisia fennel cultivation doesn't cover the local market needs. Also, fennel seeds are produced by farmers traditionally, and none of the local cultivars is named nor labelled in the market. Efforts have been deployed during the present work aiming to the characterization and valorization of genetic fennel resources. Hence, morphological diversity assessment was carried out among 62 entries of fennel acquired from different origins. Two steps principle component analysis (PCA) were applied. It came out with 7 selected cultivars of fennel representative of the initial diversity as assessed within the 62 collected fennel entries. Interestingly, among these latter, some of them exhibited particular phenotypic features linked to production traits.

1. INTRODUCTION

Fennel (*Foeniculum vulgare*) is a prominent aromatic and medicinal plant belonging to Apiaceae family. The genus *Foeniculum* is subdivided into two subspices: *Foeniculum vulgare* Mill. subsp. piperitum and *Foeniculum vulgare* Mill. subsp. *vulgare*. This latter contains three varieties, i.e., *Foeniculum vulgare* Mill. subsp. *vulgare* var dulce, *Foeniculum vulgare* Mill. subsp. *vulgare* var *vulgare* and *Foeniculum vulgare* Mill. subsp. *vulgare* var azoricum (Lopez et al., 2010). *F. vulgare* is an annual, biennial or perennial plant with erect and branching stems. At maturity, fennel plant height is in general between 50 and 200 cm (Kaurand Arora, 2010). It has 2 narrow elongated cotyledons, small and yellow flowers (Wahbaet al., 2018). Its first leaf is divided into narrow strips (Bonnier, 1920); while its much divided leaves are in filiform strips (Dheebisha and Vishwanath, 2020). The leaves are dull or glossy, dark or light. The leaves of the dulce variety are glaucescent bluish green. Vegetation is more abundant for the *vulgare* variety than for the dulce variety (Desmarest, 1978), which implies different vegetation duration between the two varieties. Generally, vegetation period could last 160-190 days for the dulce variety (Chingova, 1967). Flowering of bulbous fennel (var *azoricum*) is earlier than that of the *vulgare* variety, while bitter fennel (var *vulgare*) would flower 6 weeks later (Desmarest, 1978). *F. vulgare* is considered as a rich source of essential oils extracted mainly from leaves and seeds and which can be exploited in the food, pharmaceutical and cosmetic industries (Sayed-Ahmed, 2018). In Tunisia, it is commonly used for its seeds as a condiment as well as for its leaves.

About fennel cultivation, it is established that a dry and cold climate favors an increases production of seeds, and a temperature of 15-20°C is optimal for plant growth (Dheebisha and Vishwanath, 2020). Fennel is widely grown in temperate and subtropical regions around the world. It is grown in Africa, Asia, Europe and South America (Al-Snafi, 2018). The main fennel producing countries are India, Egypt, Turkey and China with India's dominant share of the world market exceeding 50% (Spices Board, 2008). India is still the largest producer of fennel with a production area of around 990,000 hectares, a production of 143,000 tons and a productivity of 17.1 q/ha (Anonyme, 2015). Fennel cultivation in Tunisia has remained limited to a few traditional farmers in the main production regions: Korba, Dar Chaabène, Somaa, Sfax, Kairouan, Djerba and Bizerte (Kalleli et al., 2019). In addition, the areas of condiments in Tunisia have been in continuous evolution since 2002. Indeed, this area was of 3154 ha in 2011 against 1274 ha in 2002, which denotes an increase of 147%. However, areas reserved for fennel cultivation decreased dramatically (from 306 to 163 ha during 2009-2011) despite the growing local market demand for this spice. In fact, these areas are very small compared to those intended for the cultivation of coriander (*Coriandrum sativum* L.), caraway (*Carum carvi* L.) and cumin (*Cuminum cyminum* L.) (Apia, 2013). Similarly, the cultivation of fennel spice in Tunisia has always remained practiced in a traditional way using uncharacterized genetic material. To our knowledge, in the Tunisian market, fennel seeds are provided by farmers to farmers, and no fennel variety is subscribed in the official national catalogue. In this context, the present work was carried out with the main objectives of determining the morphological characteristics of Tunisian fennel and set up a representative collection of local fennel diversity. Among these, best performing material is selected as a potential new variety.

2. MATERIAL AND METHODS

2.1. Plant material

Fennel samples were collected from fennel growers originating from the Sahel region, precisely Chrahil and Baloum regions, in addition to one sample provided by the CTAB (Centre Technique de l'Agriculture Biologique). Besides, two seeds' samples were purchased from fennel sellers in the local market, one of them is fennel imported from India, and the second one is produced in Tunisia (Table 1). Each seeds' sample is considered as a heterogeneous population. Consequently, each seed is considered to be a distinct genotype and represents a single entry.

2.2. Experimental conditions

Experimentation site is at the Regional Research Centre on Horticulture and Organic Agriculture at Chott Meriem in Sousse governorate (latitude 35°38' North; longitude 10°33' East; altitude 36m). The region is characterized by a semi-arid climate with average annual rainfall of around 230 mm, an average temperature of 18.5°C and sandy-textured soil (Kalleli et al., 2019).

Fennel seedlings were grown in nursery conditions until plantlets reached two to three leaves stage. Then, the fennel plantlets were transplanted in open field under conventional cultivation system during 2015/2016 season. The trial was organized in a single block, since each seed from the initial fennel population is considered as a unique entry. The evaluation measurements were carried out on each of these entries.

The second year trial (2016/2017) was conducted under conventional cultivation conditions using seeds harvested from the 1st trial. The experimental design adopted in the 2nd year trial corresponds to a Randomized Block Design (RBD) using 3 blocks and 10 plants per experimental unit.

Field plantation was carried out manually and

Table 1. Entries of fennel samples

Sample name	Geographic origin	Source	Pays	Sample size (entries)
CHR	Monastir	Farmer	Tunisia	5
BLMB	Sousse	Farmer1	Tunisia	15
BLMC	Sousse	Farmer2	Tunisia	11
CTAB	Sousse	CTAB	Tunisia	2
TUN	Cap Bon	Commercial	Tunisia	16
IND	Inde	Commercial	India	13

CHR : Chrahill region ; BLMB : Baloum region; BLMC : Baloum region ; CTAB : Centre Technique de l'Agriculture Biologique ; TUN : Tunisian fennel ; IND : Indian fennel.

plant spacing was 60 cm within and 160 cm between rows. Plants were watered once or twice a week depending on climatic conditions with a drip irrigation system and they were cultivated using the standard horticultural practices in the region for open field fennel.

2.3. Agronomic characterization

Based on UPOV (Union Internationale pour la Protection des Obtentions Végétales) standards (2002), quantitative and qualitative parameters of plant growth, flowering and seed production were measured. More precisely, qualitative traits were recorded at seedling stage, *i.e.* leaf curvature, intensity of green color (from very light to very dark), foliage density and plant habit. Moreover, quantitative parameters were: length of cotyledons (CotyL) (cm), leaf length (Lleng) (cm) and leaf width (LWid) (cm), main stem height at flowering stage (FlrH) (cm), main umbel appearance (Umb) (number of days after plantation: DAP), flowering start (Flr) (DAP), petiole length (PetL) (cm), main umbell diameter (UmbD)(cm) and seed maturity date (Mat) (DAP).

The measurements were carried out on all 62 entries during 1styear trial, whereas 16 plants were considered for each fennel entry during the 2nd year trial. Plant material sown during the 2nd year trial originated from the 1st selection trial where seeds were collected following a conservative multiplication procedure.

2.4. Statistical analysis

First, normal distribution was verified then ANOVA (analysis of variance) was applied to the data of morphological descriptors while considering fennel entry as a factor. Mean separation was performed by DMRT (Duncan's multiple range test) comparison of means at p=0.05.

Moreover, multivariate principal component analysis (PCA), using Varimax rotation method and Kaiser Normalization, was applied to the data set corresponding to the fennel entries. Mean values were calculated for each fennel entry based on data of quantitative traits. All statistical analyses were performed using the SPSS program, IBM Statistics 20.0 (IBM Corp. 2011).

3. RESULTS AND DISCUSSION

3.1. Agronomic diversity assessment: 1st year selection

During the first year selection, fennel collection composed of 62 entries was cultivated under conventional conditions, where each fennel entry comprised one single plant. Indeed, as starting plant material samples were collected from farmers without any previous verification of homogeneity state within each fennel population (farmer's sample or commercial sample). Hence, each fennel plant was considered as a single entry.

3.1.1. Qualitative traits

Based on UPOV guidelines, the measured qualitative traits of *F. vulgare* were: foliage habit; foliage density; leaf curvature and green color intensity.

All over the 62 fennel entries, no foliage with horizontal habit was recorded. Indeed, only upright habit (55%) and semi-upright habit (45%) were observed. The first group comprised most of the CTAB, CHR, BLMC and TUN populations, while the second group contained entries mainly from the BLMB and IND populations. Particularly, the foliage habit trait should be considered while preparing the plantation plan. Indeed, cultivars with horizontal habit need higher plant spacing. While, in the opposite plantation of cultivars with upright habit is performed with lower plant spacing. In addition, it was well established from the literature (Waskela et al., 2017) how plant spacing could significantly affect both of growth parameters and yield. Accordingly, adequate plant spacing should be applied with respect to the plant habit type in a way to provide best possible growth conditions for better harvest.

Three groups were observed based on variation of the foliage density. Group of high density foliage accounted for 55% of the fennel collection, basically included BLMB (Baloum) and TUN (Tunisian) entries. Whereas, 31% of the collection had medium foliage density and only 14% had loose foliage density. Unlikely, Lopes et al.(2009) showed the presence of a medium to low-medium density in the case of 9 Portuguese fennel accessions against a commercial accession (bitter fennel), the only one with high foliage density.

High foliage density might be a desired selection criterion, *e.g.* Florance fennel variety (var *dulce*) is known for its dense foliage and which is often valued as highly sought-after waste (Senatore et al., 2013). Furthermore, a high foliage density, besides of being under the genetic effect, can

also be favored by the use of fertilizers (Waskela et al., 2017).

Curvatured leaf phenotype showed great variability among entries from different populations. The results made it possible to highlight the presence of the three types of leaf curvature (absent, weak and strong) with a majority of plants having a strongly expressed leaf curvature (up to 45% of the population), followed by fennel plants with leaves with weakly expressed leaf curvature (31%).

The green color of fennel foliage can vary from very light green to very dark green according to UPOV standards (2002). This variation was detected among the 62 fennel entries with a predominance of medium green color (46.77%) and dark green color (37.09%). Whereas, 6.45% of the entries had a light green color, 6.45% showed a very dark green color and only 3.22% exhibited a very light green color including entries from CTAB sample. These results agree with those of Lopes et al. (2009) who showed that, among a collection of 10 fennel accessions, the medium green color of fennel leaves is predominant compared to the other intensities of the green color. However, low rates for light green, dark green and very dark green colors were found. Higher intensity of foliage green color is a good indicator of chlorophyll content, which is considered as a healthy compound for its numerous benefits (an excellent oxygen provider; an excellent acid-base regulator; an internal pH balance regulator and a powerful antioxidant) (Leventİnanç, 2011).

3.1.2. Quantitative traits

Assessment of quantitative traits during 2015/2016 season (1st year selection), came out with a morphological characterization of the fennel entries. More precisely, parameters considered in this analysis are: cotyledon length (CotyL), leaf length (LLeng), leaf width (LWid), the main umbel appearance (Umb), the flowering start (Flr), the main stem height at flowering stage (FlrH) and the seed maturity (Mat). The mean values of measured parameters are summarized in Table 2. It pointed out a high diversity level among the 62 fennel entries.

These data were further subjected to a multivariate principal component analysis (PCA), named PCA1. It came out with three principal components (PC1-1, PC1-2 and PC1-3). Table 3 shows proportional share of each trait in the two first principle components. PC-1 explained 47.64% of the total variance, and its loading was for five descriptors: LLeng, LWid, Umb, Flr, and FlrH. PC1-2 explained 17.32% of the total variance and it is accounted for Mat and negatively for CotyL. The two principal components accounted for 64.96% of the total variance.

Table 2. Mean values of descriptives traits as measured among the fennel collection

Trait	Mean ± standard deviation
CotyL (cm)	4.224±0.833
LLeng (cm)	41.411±14.465
LWid (cm)	35.938±13.216
Umb (DAP)	110.612 ±7.608
Flr (DAP)	124.387 ±4.998
FlrH (cm)	98.556±34.300
Mat (DAP)	148.741±7.910

CotyL: Cotyledon length; LLeng: leaf length; LWid: leaf width; Umb: the main umbel appearance; Flr: the flowering start; FlrH: the main stem height at flowering stage; Mat: the seed maturity; DAP: number of days after plantation.

Table 3. Total Variance and trait contribution to Principal Components of PCA1

	PCA1 Components	
	PC1-1	**PC1-2**
Variance (%)	47.641	17.317
Cumulative	**47.641**	**64.958**
Trait*		
CotyL	-0.178	**-0.495**
LLeng	**0.903**	-0.178
LWid	**0.678**	0.053
Umb	**0.932**	0.162
Flr	**0.933**	0.155
FlrH	**0.538**	-0.474
Mat	-0.005	**0.811**

**cotyledon length (CotyL), leaf length (LLeng), leaf width (LWid), the main umbel appearance (Umb), the flowering start (Flr), the main stem height at flowering stage (FlrH) and the seed maturity (Mat); Extraction Method: Principal Component Analysis; Rotation Method: Varimax with Kaiser Normalization Scores.*

Figure 1 showed fennel entries distribution on the plan (PC1-1, PC1-2) which is covering 64.96% of total variance. Three groups of fennel entries were discriminated based on their morphological traits. Description of the three PCA1 groups is given hereafter:

- Group 1 (GR1): it includes entries of Tunisian and Indian fennel characterized by short cotyledons, small leaves, short duration for the appearance of the first umbel and for flowering, medium height and seed maturity duration quite long;
- Group 2 (GR2): it comprised entries of fennel from Baloum and Chrahil regions characterized

by cotyledons from short to fairly long, large leaves, late appearance of the first umbel, late flowering, relatively medium height and long duration of seed maturity;

- Group 3 (GR3): it is made up of fennel entries belonging to the CTAB fennel sample and the $BLMC_4$ entry characterized by medium length cotyledons, medium leaf length, low leaf width, early appearance of the first umbel, short time from planting to flowering, relatively high plant height and long period of seed maturity.

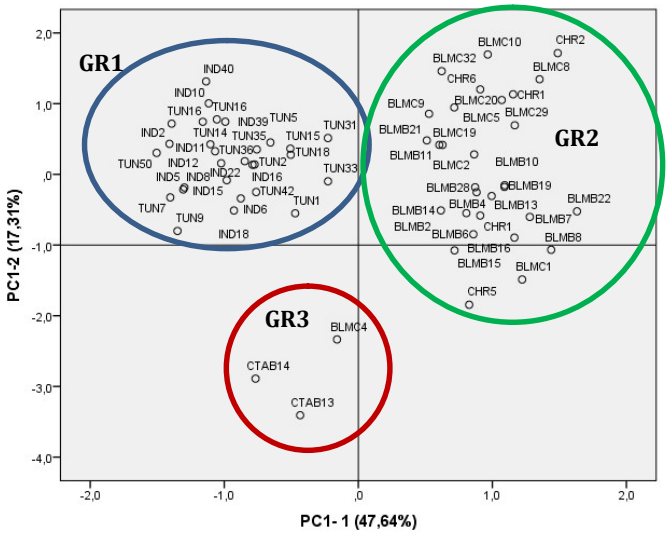

Fig. 1. Plot of first and second principal components (PC1-1 and PC1-2) of 62 fennel entries based on their respective morphological profiles (PCA1); GR1 (group 1); GR2 (group 2); GR3 (group 3)

The results of research by Sabzi et al. (2014) of Iranian fennel populations, involving 20 morphological traits, showed great diversity among studied fennel genotypes. This work came out with two doscriminated groups. Similarly, Abou El-Nasr et al. (2013) reported significant differences between three varieties of fennel (Balady, Holland and Indian) based on their morphological traits. In Tunisia, Kalleli et al. (2019) have identified 3 groups of fennel among 7 cultivars originating from different cultivation regions in Tunisia, in addition to 2 cultivars coming from two different regions in France. Their PCA analysis was also based on the study of morphological traits. Ultimately, analysis of our data allowed us to select 20 fennel entries, representative of the initial diversity occurring within the collection of 62 fennel entries, as measured through morphological parameters. Hereafter, the detailed composition of each PCA1 group giving the name of fennel sample followed by the entry number:

- Fennel entries selected from the largest group, GR1: IND_{40}, IND_{15}, IND_{18}, TUN_{50}, TUN_{9}, TUN_{31} and TUN_{36};

- Fennel entries selected from GR2: CHR_5, CHR_2, CHR_1, $BLMC_{32}$, $BLMC_9$, $BLMC_5$, $BLMB_{22}$, $BLMB_2$, $BLMB_{11}$ and $BLMB_{10}$;

- Fennel entries selected from GR_3: $CTAB_{13}$, $CTAB_{14}$ and $BLMC_4$.

3.2. Agronomic diversity assessment: 2nd year selection

Conservative multiplication was applied during 1st year trial to produce fennel seeds from the 62 fennel entries. Thus, 62 families of fennel seeds were generated. The 1st year selection ended up with 20 fennel entries representative of the initial phenotypic variation as observed among the collection of 62 fennel entries. Only fennel families from the 20 pre-selected genotypes were grown for a 2nd year selection during 2016-2017 season. The 20 selected fennel families comprised 3 families from the IND sample, 4 families from the TUN sample, 8 families from the BLM (7 families from farmer 1 and 1 family from farmer 2), 3 families from the CHR sample, and 2 families from the CTAB sample.

3.2.1. Qualitative traits

The same morphological parameters used in the 1st year were evaluated during the 2nd year trial. Regarding the foliage habit, each fennel family showed identical phenotype among its members. Diversity of the foliage habit was observed between fennel families, and two groups of families were identified: one group characterized by a semi-upright habit including 15 families (75%) and a second group of upright habit comprised of 5 families (25%) (Table 4). From the literature, similar results were reported by Lopes et al. (2010). They found that among a total of 9 fennel populations only one commercial population had the upright habit type and 8 expressed a semi-upright habit.

Foliage density type among the 20 fennel families is summarized in Table 5. 70% of the fennel families (14) had medium foliage density, 2 of them with a loose phenotype, and 4 families displayed highly dense foliage density phenotype.

About leaf curvature type, each fennel family comprised member's whith the same phenotype. On the other hand, two phenotypes were distinguished, *i.e.*, strong phenotype, including 45% of the fennel families; and weak phenotype harboring 55% out of the 20 selected fennel families (Table 6).

Table 4. Plant habit phenotype as expressed by the 20 selected fennel families

Plant habit type	Fennel families
Semi-upright	$BLMC_9$, CHR_2, TUN_9, $BLMC_{32}$, TUN_{50}, TUN_{31}, IND_{15}, $BLMB_{22}$, IND_{18}, $BLMB_{11}$, $BLMB_{10}$, TUN_{36}, $CTAB_{14}$, $BLMB_2$ & $BLMC_4$
Upright	$BLMC_5$, $CTAB_{13}$, IND_{40}, CHR_5, & CHR_1

Table 5. Foliage density phenotype as expressed by the 20 selected fennel families

Foliage density type	Fennel families
Loose foliage	IND_{15} & $CTAB_{13}$
Medium density	$BLMC_9$, $BLMC_5$, $BLMC_{32}$, TUN_{50}, TUN_{31}, $BLMB_{22}$, IND_{18}, IND_{40}, $BLMB_{11}$, $BLMB_{10}$, TUN_{36}, $CTAB_{14}$, $BLMB_2$ & $BLMC_4$
High density	CHR_2, TUN_9, CHR_5 & CHR_1

Table 6. Leaf curvature type phenotype as expressed by the 20 selected fennel families

Leaf curvature type	Fennel families
Strong	$BLMC_9$, CHR_2, TUN_9, $BLMC_{32}$, TUN_{31}, $CTAB_{13}$, IND_{18}, CHR_5, & CHR_1
Weak	$BLMC_5$, TUN_{50}, IND_{15}, $BLMB_{22}$, IND_{40}, $BLMB_{11}$, $BLMB_{10}$, TUN_{36}, $CTAB_{14}$, $BLMB_2$ & $BLMC_4$

Table 7. Intensity of foliage green color phenotype as expressed by the 20 selected fennel families

Foliage green color intensity	Fennel families
Very dark	CHR_2, TUN_9, CHR_5 & CHR_1
Medium	$BLMC_9$, $BLMC_5$, $BLMC_{32}$, TUN_{50}, TUN_{31}, IND_{15}, $BLMB_{22}$, IND_{40}, $BLMB_{11}$, $BLMB_{10}$, TUN_{36}, $CTAB_{14}$, $BLMB_2$ & $BLMC_4$
Very light	$CTAB_{13}$ & IND_{18}

The green color of fennel foliage study came out with three groups of fennel families (Table 7). Again, the main group was characterized by a medium green color phenotype counting for 75% of the fennel families. Whereas, very dark phenotype and very light phenotype included respectively 4 and 2 fennel families only.

3.2.2. Quantitative traits

Investigation of the same quantitative morphological descriptors as during the 1st year study was carried out, namely: the length of the cotyledons, the width and length of the leaf, the time of appearance of the main umbel and its flowering, the plant height and time of seed maturity. Additionally, traits of petiole length of the first leaf and the diameter of the main umbel were added to better highlight the existing diversity among fennel families.

Data collected were subjected to DMRT analysis and subsequent results are summarized in Table 8. All measured parameters displayed significant differences between fennel families. For instance, the TUN_{50} family had the longest cotyledon (6.91 cm). While, $BLMC_{32}$ and IND_{18} families displayed the lowest values (4.6 and 4.84 cm, respectively).

Table 8. Mean values of descriptive traits as measured among selected fennel families

Fennel family	CotyL (cm)	PetL (cm)	LLeng (cm)	LWid (cm)	Umb (DAP)	Flr (DAP)	FlrH (cm)	UmbH (cm)	Mat (DAP)
$BLMC_9$	5.35±0.70 b	4.86±0.46 b	80,87±4.93 j	76.5±4.66 h	115.75±1.61 f	131.43±2.15 g	40.81±3.65 a	12.76±0.60 de	160.37±0.80 l
$BLMC_5$	5.7±0.68 bc	5.53±0.5 c	59.62±7.61 i	53.68±7.49 f	111.31±1.35 d	127.06±4.28 e	65.68±4.06 b	16.03±0.4 hi	154.37±0.95 i
CHR_2	6.23±0.53 def	6.68±0.53 g	49.12±4.33 efgh	49.12±4.82 e	105.56±1.15 c	121.06±1.38 d	140±2.6 h	13.43±1.01 f	140±0.00 d
TUN9	6.47±0.90 fgh	6.26±0.64 ef	49.68±4.09 fgh	50.62±3.24 ef	105.81±1.10 c	121.18±1.27 d	141.93±3.10 h	13.36±0.57 f	144±0.00 f
$BLMC_{32}$	4.6±0.48 a	4.03±0.55 a	81.06±4.20 j	77.31±4.42 h	116.18±1.51 f	131.75±2.14 g	40.93±3.41 a	13.19±0.59 ef	167±0.00 m
TUN_{50}	6.91±0.44 h	6.22±0.5 ef	27.25±5.14 b	23.68±4.79 b	69.87±1.54 a	82.43±2.18 a	135.37±2.55 g	15.31±0.87 g	127.5±2.58 a
TUN_{31}	6.01±0.47 cdef	4.89±0.59 b	81.5±3.05 j	77.62±2.12 h	115.12±1.40 f	129.62±2.89 f	38.87±3.24 a	12.52±0.41 bcd	159.93±1.43 l
IND_{15}	5.96±0.49 cde	4.94±0.50 b	22.81±4.38 a	20.68±4.64 a	74.12±3.63 b	86.62±4.08 b	87±5.48 c	11.01±0.81 a	127±0.00 a
$CTAB_{13}$	5.68±0.83 bc	5.88±0.71 cde	46.06±4.26 de	49.75±6.29 e	75.12±2.70 b	96.75±1.91 c	173±6.98 i	16.4±0.85 i	157±0.00 k
$BLMB_{22}$	5.7±0.66 bc	6.06±1.05 def	45.87±2.96 de	43.18±2.83 d	111.93±1.38 de	127.43±1.26 e	125.31±3.41 de	12.63±0.49 cd	140±0.00 d
IND_{18}	4.84±0.40 a	4.87±0.47 b	80.75±3.97 j	76.43±3.44 h	115.87±1.31 f	130.68±2.02 fg	39.81±5.30 a	12.43±0.44 bcd	168±0.00 n
IND_{40}	6.4±0.53 efg	5.97±0.65 cdef	61.87±5.54 i	57.12±5.11 g	111.25±1.29 d	127.06±4.32 e	63.81±5.55 b	15.73±0.59 h	156±0.00 j
CHR_5	6.4±0.55 efg	6.3±0.69 ef	50.68±2.35 gh	51.62±1.85 ef	105.43±1.03 c	121.43±1.20 d	139.12±1.45 h	12.61±0.84 cd	145±0.00 g
CHR_1	6.36±0.59 efg	6±0.58 c.def	51±3.30 h	51.68±4.12 ef	105.18±0.98 c	121.25±1.29 d	141.75±3.31 h	12.16±0.40 bc	146±0.00 h
$BLMB_{11}$	5.43±0.64 b	5.7±0.47 cd	47.62±3.05 defg	44.62±2.57 d	112.12±1.45 de	127.31±1.30 e	123.81±3.29 d	12.22±0.56 bc	139±0.00 c
$BLMB_{10}$	5.75±0.67 bcd	5.65±0.73 cd	46.75±3.35 def	44.25±3.47 d	112.18±1.37 de	127.25±1.23 e	123.75±3.21 d	12.33±0.55 bcd	144±0.00 f
TUN_{36}	5.76±0.67 bcd	5.81±0.59 cde	45.06±4.20 d	42.37±4.12 d	112.06±1.38 de	127.43±1.26 e	123.31±2.67 d	12.17±0.39 bc	143±0.00 e
$CTAB_{14}$	5.64±0.62 bc	5.61±0.70 cd	45.56±3.34 d	43.18±3.33 d	112.12±1.36 de	127.37±1.54 e	125.93±3.23 de	12.04±0.40 b	145±0.00 g
$BLMB_2$	5.58±0.68 bc	5.65±0.40 cd	47.06±2.67 def	44.62±3.18 d	112.81±1.83 e	127.12±1.58 e	126.87±2.57 e	12.05±0.37 b	144±0.00 f
$BLMC_4$	6.75±0.57 gh	6.43±0.76 f	30.68±4.36 c	27.75±4.90 c	69.81±1.72 a	84±2.30 a	132.68±3.38 f	16.02±0.44 h i	129.37±0.5 b

**cotyledon length (CotyL), leaf length (LLeng), leaf width (LWid), the main umbel appearance (Umb), the flowering start (Flr), the main stem height at flowering stage (FlrH), petiole length (PetL), umbell diameter (UmbD) and the seed maturity (Mat) ; means followed by different letters in the same column were significantly different (P<0.05) using Duncan test.*

In addition, the petiole length of the first leaf varied between 4.03 and 6.68 cm. First leaf petiole length of our plant material was relatively higher than corresponding data from the literature. Indeed, Kalleli et al. (2019) reported a variation of first leaf petiole length going from 1.06 to 4.52 cm. It was from 0.3 to 2.1 cm according to Lopes et al. (2009). Such variation could be explained by either genetic and/or environmental reasons (Kallali et al., 2019).

About length and width of the leaf, maximum values were recorded in the case of $BLMC_{32}$ and IND_{18}, which had the shortest cotyledons, besides TUN_{31} and $BLMC_9$. Whereas, IND_{15} showed the minimum value.

Flowering related traits, namely the time of the appearance of the main umbel as well as the time of flowering, displayed significant differences between fennel families (Table 8). TUN_{50} and $BLMC_4$ fennel families were the earliest, showing an average maturity period of 70 DAP. In the opposite, referring to appearence of the first umbel, all BLMC and BLMB families, except $BMLC_4$, were classified as late. These two flowering related traits showed identical profile among the fennel families over the two years trials.

Assessment of the flowering time of the umbels, hilighted the occurrence of 7 homogeneous groups. The first group included the early families (TUN_{50} and $BLMC_4$). While, the second and third groups comprised a single family each, IND_{15} and $CTAB_{13}$, respectively. Group 4 contained the largest number of fennel families with an average of 127 DAP. TUN_{31} and $BLMC_9$ were the latest families to reach flowering of the umbels: they required an average of 130 DAP.

Plant height assessment at flowering stage detected the occurrence of significant differences ($P<0.05$) between the fennel families. The shortest plants were from $BLMC_9$, $BLMC_{32}$, TUN_{31} and IND_{18} which had the shortest average values with 40.81, 40.93, 38.87 and 39.81 cm of height means respectively. Maximum values were measured in the case of CHR (CHR_1 and CHR_5 together) and TUN_9 families with 121 DAP and $CTAB_{13}$ family with 127 DAP.

Largest umbels were observed within $CTAB_{13}$, $BLMC_5$ and $BLMC_4$ families, while IND_{15} exhibited the smallest umbels. This latter was also characterized by a small leaf; a 74 DAP period for the appearance of the main umbel and an early flowering. Particularly, Tunisian fennel families displayed quite high umbel diameter values ranging from 12.17 to 16.4 cm. Interestingly, this finding can be favorable for yield enhancement. Accordingly, Kalleli et al. (2019) found values varying between 10 and 17.76 cm of umbel diameter within fennel cultivars harvested from different Tunisian regions.

To conclude, many of the Tunisian fennel families, characterized by a short leaf, an early duration of appearance of the main umbel and an early flowering duration, matured first (TUN_{50}, $BLMC_4$, $BLMB_{11}$, $BLMB_{22}$ and CHR_2 with 127, 129, 139, 140 and 140 DAP, respectively). While, IND_{18} family had the latest umbel maturity period (168 DAP), which had particular morphological traits, *i.e.* short cotyledons, low plant height and late flowering.

Mostly, correlation matrix (Table 9) indicated highly significant correlation levels ($r>0.5$). Most important correlations are those associating leaf traits (LLeng and LWid) to umbel and fruit traits (Umb, Flr and Mat). It is well established that these latter are strongly associated with seed yield. Subsequently, they could potentially be used for high yield selection (Kalleli et al., 2019). Particularly, best correlation score ($r=0.994$) associated Umb (time of appearance of the first umbel) to Flr (time of flowering). However, the lowest correlation score ($r=-0.748$) related FlrH (plant height at flowering stage) to LLeng (leaf length).

Moreover, multivariate PCA analysis of 2nd year selection (PCA2) was applied to the 20 selected fennel families using data recorded of the 9 morphological traits. Three principle components (PC2-1, PC2-2 & PC2-3) were obtained (Table 10). PC2-1, PC2-2 and PC2-3 respectively explained 63.23, 15.16 and 12.49% of the total variance. PC2-1 was positively corrolated to most of the traits, *i.e.* LLeng, LWid, Umb, Flr and Mat and negatively highly related to CotyL, PetL and FlrH. Besides, PC2-2 accounted for 15.16% of the total variance with a positive contribution of UmbD.

Figure 2 displayed fennel entries distribution on the plan (PC2-1, PC2-2) which is covering 78.4% of total variance. Six groups of fennel entries were discriminated based on their morphological traits. Description of each group features are as follow:

- Group 1: it comprised $BLMC_{32}$, $BLMC_9$, TUN_9 and IND_{18} families displaying large leaves, late appearance of the main umbel, late flowering, short plant height, and medium diameter of the first umbel;

Table 9. Correlation matrix between the 9 morphological descriptors of fennel families grown under conventional cultivation conditions

	CotyL	PetL	LLeng	LWid	Umb	Flr	FlrH	Mat	UmbD
CotyL	1	0.773	-0.6	-0.583	-0.55	-0.565	0.513	-0.652	0.349
PetL	0.773	1	-0.616	-0.574	-0.332	-0.317	0.79	-0.601	0.336
LLeng	-0.6	-0.616	1	0.991	0.708	0.723	-0.748	0.929	-0.082
LWid	-0.583	-0.574	0.991	1	0.69	0.715	-0.67	0.94	-0.088
Umb	-0.55	-0.332	0.708	0.69	1	0.994	-0.441	0.601	-0.447
Flr	-0.565	-0.317	0.723	0.715	0.994	1	-0.406	0.645	-0.401
FlrH	0.513	0.79	-0.748	-0.67	-0.441	-0.406	1	-0.627	0.111
Mat	-0.652	-0.601	0.929	0.94	0.601	0.645	-0.627	1	0.06
UmbD	0.349	0.336	-0.082	-0.088	-0.447	-0.401	0.111	0.06	1

**cotyledon length (CotyL), leaf length (LLeng), leaf width (LWid), the main umbel appearance (Umb), the flowering start (Flr), the main stem height at flowering stage (FlrH), petiole length (PetL), umbell diameter (UmbD) and the seed maturity (Mat).*

Table 10. Total Variance and trait contribution to Principal Components of PCA2

	PCA2 principle components		
	PC2-1	**PC2-2**	**PC2-3**
Variance (%)	63.23	15.16	12.49
Cumulative variance (%)	**63.23**	**78.4**	90.89
Traits*			
LLeng	0.944	0.217	0.171
LWid	0.924	0.204	0.213
Mat	0.89	0.327	0.178
Flr	0.816	-0.41	0.377
Umb	0.809	-0.444	0.334
CotyL	-0.781	0.103	0.348
FlrH	-0.765	-0.301	0.313
PetL	-0.742	-0.142	0.634
UmbD	-0.299	0.826	0.378

**cotyledon length (CotyL), leaf length (LLeng), leaf width (LWid), the main umbel appearance (Umb), the flowering start (Flr), the main stem height at flowering stage (FlrH), petiole length (PetL), umbell diameter (UmbD) and the seed maturity (Mat); Extraction Method: Principal Component Analysis; Rotation Method: Varimax with Kaiser Normalization Scores.*

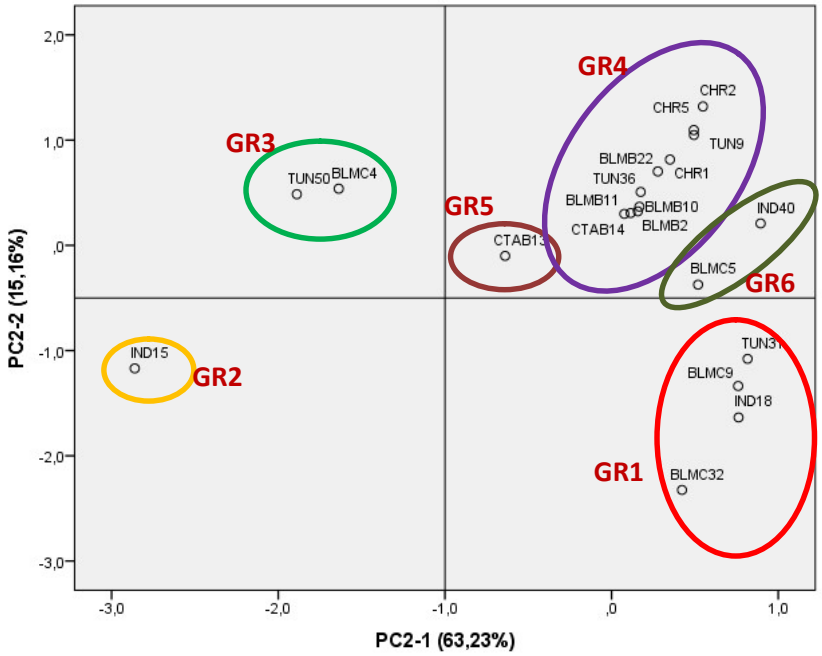

Fig. 2. Plot of first and second principal components of PCA2 (PC2-1 and PC2-2) of pre-selected 20 fennel families based on their respective morphological profiles. (GR1 to 6: group 1 to 6)

- Group 2: it included only IND_{15} family characterized by a medium size of cotyledon and petiole, a small leaf size, a small umbel diameter, a short plant height, an early appearance of the first umbel, an early flowering and an early seeds maturity;
- Group 3: it comprised TUN_{50} and $BLMC_4$ families which had the longest cotyledons, short leaves, fairly increased plant height, most early appearance of the main umbel; most early flowering and most early seeds maturity;
- Group 4: it consisted of families $BLMB_{11}$, $BLMB_{22}$, $BLMB_2$, $BLMB_{10}$, TUN_{36}, TUN_9, $CTAB_{14}$, CHR_1, CHR_2 and CHR_5, which had intermediate values levels for all the traits;
- Group 5: it contained the $CTAB_{13}$ family, characterized by the highest plants;
- Group 6: it involved families $BLMC_5$ and IND_{40} with a large main umbel diameter.

Ultimately, thanks to this selection step, 7 fennel families were picked up: $BLMC_{32}$ (GR1), IND_{15} (GR2), TUN_{50} (GR3), CHR_5 and $BLMB_{11}$ (GR4), $CTAB_{13}$ (GR5) and $BLMC_5$ (GR6).

4. CONCLUSION

The present work gave evidence on the phenotypic diversity of a local Tunisian fennel collection. Detailed characterization gave enough data to enable us to select genetic material showing interisting production traits, *i.e.*, the time of appearance of the main umbel and its flowering, the plant height and time of seed maturity.

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