

A comparative study of morphological characterization of Tunisian accessions of Chili pepper (*Capsicum frutescens* L.)

Kaouther ZHANI¹, Wissem HAMDY¹, Sami SEDRAOUI², Rami FENDRI¹, Oula LAJIMI¹, Chérif HANNACHI¹

¹ Higher Institute of Agronomy, 4042, Chott Mariem, Tunisia, Sousse University,

² Faculty of Sciences of Bizerte, Tunisia, Carthage University

Abstract - In order to protect biodiversity and preserve local germplasm, five local accessions of chili pepper (*Capsicum frutescens* L.): Tébourba, Somâa, Korba, Awled Haffouz and Souk Jedid, were collected from Manouba, Nabeul and Sidi Bouzid states (Tunisia) and cultivated in the experimental station of Higher Institute of Agronomy, Chott Mariem, Sousse (Tunisia) for their morphological characterization. Results showed that the pepper accessions were significantly different ($p < 0.01$) in many characters studied. Souk Jedid accession produced plants with erect and short habit, small and lanceolate leaves and grouped flowers whereas the rest of accessions have dichotomous branching with bushy secondary stems, large and oval leaves and solitary flowers. The fruits had enrobing calyx, narrow triangular shape, dark red color at maturity and a smooth to slightly wrinkled surface in addition they are pungent but difference was observed in attitude, situation of pericarp at basal part, surface texture, glossiness

Key Words: *Capsicum frutescens*, fruits, flowers, genotypes, leaves, morphology.

1. Introduction

Pepper is a member of the *Solanaceae* family and *Capsicum* genus. This genus ranged for 20 to 30 species [1], from which five of them have become domesticated: *Capsicum annum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum pubescens* and *Capsicum baccatum* [2]. These species have the same number of chromosomes ($2n = 2x = 24$) [3]. Pepper has important roles in various aspects of economy, food and pharmacy. Its fruits, used as spice, vegetable and herbal remedy, are known by their highest vitamin C content among all vegetables [4] and their antioxidant characteristics [5]. Many researchers studied the phenotypic variability of pepper regarding plant and fruit characteristics in order to select those which are desirable in the initiation of a pepper breeding program.

This variability is firstly studied according to the international plant genetic resources institute (IPGRI) descriptor. Recently, the descriptor of the international union for the protection of new varieties of plants (UPOV) is the widely morphological descriptor used to describe the genotypes belonging to several vegetable crops other than pepper [6] such as eggplant [7], zucchini [8], strawberry [9], squash [10] and tomato [11]. The genotypic yield difference is also used by plant breeders to carry out the selection process by selecting simpler traits such as number of branches, number of flowers, fruit number, fruit size and fruit yield [12]. In Tunisia, pepper is widely grown in all regions both on open air and under greenhouse and occupies the fourth largest area planted by gardening. In 2013, the area allocated to this crop reached 21,200 ha with a national yield of 18.1 t/ha [13]. In fact, this vegetable is very appreciated by Tunisian consumer's especially pungent varieties which are used fresh to prepare different dishes, dried (spice) or pickled. The majority of cultivated varieties are hybrids belonging to the genus *Capsicum annum* and estimated to be 34, all are officially allowed by Agriculture Minister [14]. The varieties belonging to *Capsicum frutescens* or chili pepper are local varieties, named "Baarbid". They are mainly used for pickling or for preparing the famous "Harissa" by industry (45000 ha are designated in 2012 for pepper transformation to Harissa [14]. Korba (state of Nabeul governess) is the famous and the major producer of chili pepper in Tunisia. Nevertheless, this type of pepper is cultivated in other regions and no study was done about the morphology and the production of the accessions cultivated there. Therefore, for the first time, the present research studies the difference between morphological characterizations of local Tunisian chili pepper.

Thus, five accessions of chili pepper (*Capsicum frutescens* L.) are collected from different regions in Tunisia: Tébourba (Tb), Somâa (Sm), Awled Haffouz (Az), Souk Jedid (Sj) and Korba (Kb). The morphology of plant and fruit was studied by UPOV descriptor on an open air culture

2. Material and methods

2.1. Experimental site

Research was conducted in the experimental station of Higher Institute of Agriculture of Chott Mariem (Tunisia). This region belongs to semi-arid bioclimatic stage characterized by sweet winter and cool summer. The mean maximum and minimum temperature are 11.4°C (January) and 28°C (August) respectively. The annual rainfall recorded is above 300 mm. Relative humidity ranges from 20 to 80%. Direction of wind is North and North-Ouest. The soil is sandy clay limestone (Hamdi, 2008) and water is characterized by a neutral pH (pH=7) and salinity of 1g/l. The site into which the trial was done was previously cultivated with cucumber.

2.2. Experimental material

Five local accessions of chili pepper were included in this study. They were collected from three Tunisian regions: Manouba, Cap Bon and Sidi Bouzid. Two of them came from two locations in Cap Bon; Somâa (Sm) and Korba (Kb), two were from the state of Sidi Bouzid: Awled Haffouz (Az) and Souk Jedid (Sj) and the fifth was collected from Tébourba (Tb) in Manouba .

2.3. Experiment setup

The morphological characterization was done on open air from April to November 2010. Pepper seeds were sown on field on April 15th at 7 leaves stage, plants were transplanting to field at a density of 2 plants/m². Irrigation and fertilization were applied as needed. "Désogerme SP Végétaux" (200 ml/hl) was utilized against powdery mildew. The experiment was completely randomized design with three replications of each accession per plot.

2.4. Data collection

The characterization was based on 52 descriptors with 34 morphological traits (Table 1) proposed by the International Union for the protection of new varieties of plants, guidelines for *Capsicum* [15] used for describing the plant, flowers, leaves and fruits.

2.5. Data Analysis

SPSS software 13.00 was used to analyze the data and Duncan's multiple range test was used to separate the means at 5% (p<0.05).

Table 1. Morphological characters in study

Plant characters
-Anthocyanin coloration of hypocotyl : 1 (present), 9 (absent)
-Habit: 1 (upright), 2 (semi-upright), 3 (prostrate)
-Length of stem: 1 (short), 5 (medium), 9 (long)
-Shortened internode (in upper part): 1 (present), 9 (absent)
-Anthocyanin coloration of nodes: 1 (present), 9 (absent)
-Intensity of anthocyanin coloration of nodes: 1 (very weak), 3 (weak), 5 (medium), 7 (strong), 9 (very strong)
-Hairiness of stem nodes: 1 (absent or very weak), 3 (weak), 5 (medium), 7 (strong), 9 (very strong)
Leaf characters
-Length of blade (cm)
-Width of blade (cm)
-Intensity of green color : 1 (very light), 3 (light), 5 (medium), 7 (dark), 9 (very dark)
-Shape : 1 (lanceolate), 2 (ovate), 3 (broad elliptic)
-Undulation of margin: 1 (absent or very weak), 3 (weak), 5 (medium), 7 (strong), 9 (very strong)
-Blistering : 1 (very weak), 3 (weak), 5 (medium), 7 (strong), 9 (very strong)
-Glossiness : 1 (very weak), 3 (weak), 5 (medium), 7 (strong), 9 (very strong)
Inflorescence characters
-Anthocyanin coloration in anther : 1 (present), 9 (absent)
-Corolla color: 1 (white), 9 (not white)
-Peduncle attitude : 1 (erect), 2 (semi-drooping), 3 (drooping)
Fruit characters
-Color before maturity: 1 (greenish white), 2 (yellow), 3 (green), 4 (purple)
-Intensity of color: 1 (very light), 3 (light), 5 (medium), 7 (dark), 9 (very dark)
-Anthocyanin coloration: 1 (present), 9 (absent)
-Color in mature stage: 1 (yellow), 2 (orange), 3 (red), 4 (brown), 5 (green)
-Intensity of color (at maturity): 1 (light), 2 (medium), 3 (dark),
-Attitude : 1 (erect), 2 (horizontal), 3 (drooping)
-Shape in longitudinal section: 1 (oblate), 2 (circular), 3 (cordate), 4 (square), 5 (rectangular), 6 (trapezoidal), 7 (moderately triangular), 8 (narrowly triangular), 9 (hornshaped)
-Shape in cross section (at level of placenta): 1 (elliptic), 2 (angular), 3 (circular)
-Situation of pericarp at basal part: 1 (absent or very weak), 3 (weak), 5 (medium), 7 (strong), 9 (very strong)
-Situation of pericarp excluding basal part: 1 (absent/very weak), 3 (weak), 5 (medium), 7 (strong), 9 (very strong)
-Texture of surface: 1 (smooth or very slightly wrinkled), 2 (slightly wrinkled), 3 (strongly wrinkled)
-Glossiness : 1 (very weak), 3 (weak), 5 (medium), 7 (strong), 9 (very strong)
-Stalk cavity : 1 (present), 9 (absent)
-Shape of apex: 1 (very acute), 2 (moderately acute), 3 (rounded), 4 (moderately depressed), 5 (very depressed)
-Number of locules : 1 (predominantly two), 2 (equally two and three), 3 (predominantly three), 4 (equally three and four), 5 (predominantly four and more)
-Calyx aspect: 1 (non enveloping), 2 (enveloping)
-Pungency (observed by tasting the pepper in the placenta area): 1 (pungent) 9 (non pungent)

3. Results

The morphological characterization of the Tunisian chili pepper studied showed a significant difference between all the accessions in all growth stage of the plant. At seedling stage, the anthocyanin coloration of hypocotyl was observed in all accessions (Table 2).

At plant stage, the characterization concerned all the organs of the pepper plant and results demonstrated that *Sj cv* was distinguished by the presence of shortened internode in upper part which appears after the first branching of the main axis making the growth of the main stem ending in a bunch of flowers. This character impacted on the habit and the height of the stem making it to have an erect habit and the highest stem height. For the other accessions, shortened internodes in upper part are absent and the plant had a prostrate habit (Figure 1) and a medium stem.



Erect habit (*Sj cv*)

Prostrate habit (*Tb cv*)

Figure 1. Plant habit of Tunisian chili pepper accessions

For hairness and anthocyanin coloration, they were observed at the nodes of all the accessions in study (Figure 2), but their intensity was variable. *Sj cv* is distinguished by a medium hair and strong pigmentation while nodes of other accessions were moderately pigmented and rich in hair.



Figure 2. Anthocyanin coloration on nodes (*Tébourba cv*)

Regarding the leaves characteristics, a difference has been noted between accessions. The shape of leaf (Figure 3) was ovate (*Tb, Sm, Kb* and *Az cv*) or lanceolate (*Sj cv*). The lowest length (6.65 cm) and width of blade (2.7 cm) were observed in *Sj cv* whereas *Tb cv* was regarding the highest length (10.51 cm) and width (4.47) of blade (Table 2). Also, only *Sm cv* had medium undulation of margin and weak blistering. For glossiness, it was weak for all accessions leaves (Table 2).

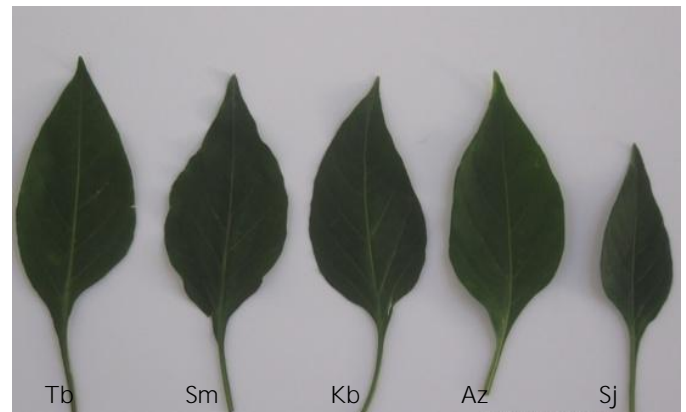


Figure 3. Leaves of Tunisian chili pepper accessions

Based on characterization by flower morphology, observation showed that flowers of all accessions consisted of white to yellowish-white (*Sj cv*) corolla, green calyx, yellowish pistil and yellow anther with anthocyanin coloration (Figure 4). The difference was noted in peduncle attitude which was erect (*Sj cv*), semi-drooping (*Kb* and *Sm cv*) and drooping (*Tb* and *Az cv*). Also, a difference in the number of flowers per axil was remarkable: *Sj cv* was characterized by a grouped inflorescence where flowers are issued in the form of clumps each with an average of 7 flowers. Other accessions showed a solitary flower.

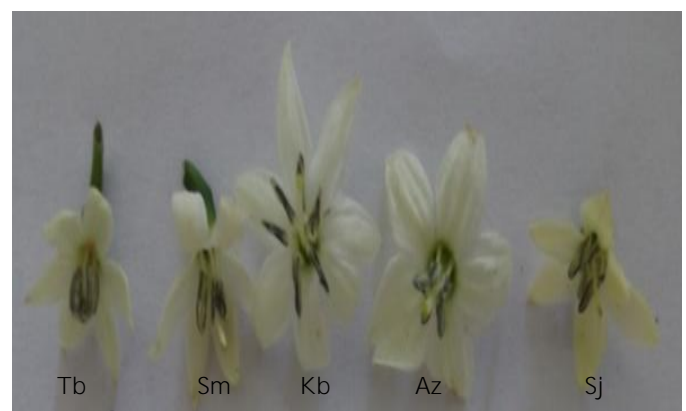


Figure 4. Flowers of Tunisian chili pepper accessions

The fruit characterization showed that immature fruits of all accessions have medium (Tb and Sj cv) to dark (Sm, Kb, Az cv) green color with anthocyanin pigmentation (Figure 5). Before maturity, in all genotypes studied, the color of fruits turns to the dark red and fruits have narrowly triangular shape in longitudinal section, circular shape in cross section (at level of placenta), very acute shape of apex and enveloping calyx (Figure 6). The sinuation of pericarp excluding basal part and stalk cavity are absent. Whereas, the difference was noted in others characters.



Figure 5. Immature fruits of Tunisian chili pepper accessions



Figure 6. Mature fruits of Tunisian chili pepper accessions

Three attitudes (Figure 7) are observed: drooping (Tb and Az cv), erect (Sj cv) and horizontal (Kb, Sm cv). Sinuation of pericarp at basal part was found to be very strong (Tb cv), medium (Sm and Sj cv) and weak (Kb and Az cv). Texture of surface was slightly wrinkled (Tb cv) or very slightly wrinkled (Sm, Kb, Az and Sj cv).

For fruit glossiness, it is medium (Tb cv), strong (Sm, Kb and Az cv) or very strong (Sj cv). By doing a longitudinal section of fruit, it was observed the presence of two locules in the fruits of all accessions. The test of Pungency, determinate by tasting the pepper flesh together with the locules, in the placenta area, was positive for all accessions.

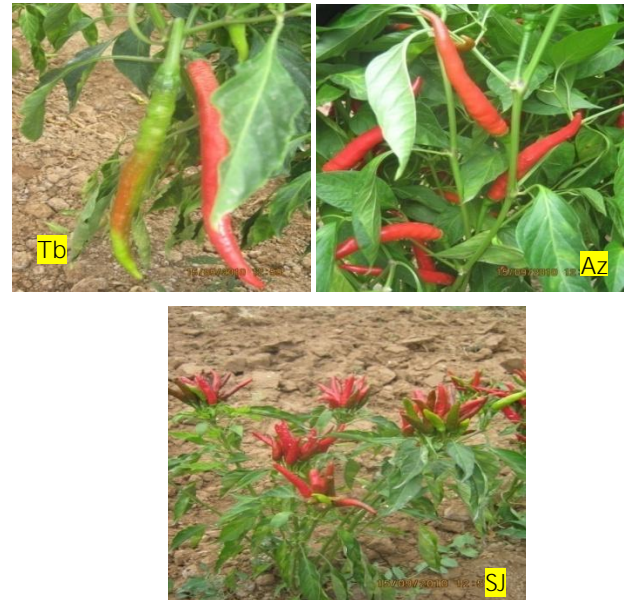


Figure 7. Fruits attitude of Tunisian chili pepper: drooping (Tb cv), erect (Sj cv) and horizontal (Az cv).

4. Discussion

In this research, the morphological characteristics and agronomic performance of five Tunisian Chili pepper studied showed a significant genotypic difference. The difference began from seedling stage with the first trait considered as useful grouping characteristics [15] and as an index of purity or not of the genotype [16]; it is the anthocyanin coloration of hypocotyl. At plant stage, this violet color was also observed on the nodes of the stems in addition to the hairs. According to Adetula and Olakojo [17], the hairs at the nodes are one of the vegetative characters used for the morphological characterization of pepper cultivars and thus, they noted the absence of the hair in 14 accessions and the presence in 19 accessions. The plant habit is determined by the bifurcation of the main stem [18]. Indeed, this stem is divided from the crown into several secondary stems and gives the plant an erect and short habit (Sj cv) or two secondary stems born away collar and give the plant a prostrate habit with high height of the plant; a typical habit of pepper [18] where the main stems grow indeterminately, one or two flowers develop per node and shortened internodes never develop. The same result was found in morphology study of 33 Nigerian accessions pepper [17] whose growth habit were prostrate (ATO 99 - 425), compast (OAB99-

440) or erect (84-175 NH). This descriptor is important in terms of crop management because it can help in terms of defining the area for each plant, harvesting, weed control and feasibility to calculate the volume of chemical spray [19]. Similar result genetic variation was also observed in height of stem on 48 Turkish genotypes of *Capsicum annuum* [20] and 40 accessions Basilian *Capsicum baccatum* [21]. In addition, accessions showed a great polymorphism that affects all organs of the plant. Indeed, the leaf is either oval or lanceolate with different dimensions. This heterogeneity in the form of sheet was also reported in other accessions of chili [22]. For flowers, all accessions have the same form and color. The color of the corolla can be used to characterize the cultivated and wild relatives of pepper [19]. However, corolla color is less suitable when the variation of the flower color of more distant wild species is described [23]. Also, the color of the anther is seen as a key descriptor for the genus *Capsicum* as it is highly discriminatory among accessions [24]. However, the difference is noted in number of flowers per axil where flowers are either grouped in form of clumps or solitaires. The last type is the typical flowering of the species *Capsicum*: (one flower per axil) located at each node, intersection of two branches [25]. Similar results [19] affirmed that the number of flowers per node varies from one flower (35 accessions), two flowers (9 accessions) to three flowers (12 accessions). Also, only 27% of these accessories have yellow anthers and 34% have a white corolla. The characterization of fruits was done at immature and mature stage and an important difference between accessions was also remarkable. The same result was found in Slovakia for shape [26], in Indonesia for color [27], in Thailand for attitude [28] and in Korea for texture of surface [29]. According to Pickersgill [30], calyx description is greatly important in differentiating *C. chinense* from *C. frutescens*, since it is present in the first and absent in the second species, and it is used to separate these two species from each other and even between accessions of the same species [29]. The typical red color of fruit at mature stage is related to transformation of chloroplasts into chromoplasts [31]. This transformation resulted in the gradual decline [32] or disappearance of lutein [33], the most abundant carotenoid in green immature fruit of pepper. Simultaneously, the biosynthesis of capsanthin is activated [34] giving the typical red color in red mature pepper fruit [35].

To explain the variability in the characteristics of the fruit, several hypotheses have been put. Bosland and Votava [1] suggest that during the fruit development, size is dependent on cell elongation during anthesis and post-anthesis whereas fruit shape is influenced by cell division that takes place in stage pre-anthesis. Later, researchers [36] affirm that this noted variability is primarily genetic related to gene "*fs 3.1*" located on chromosome 3 controlling the shape of the fruit. As for the size of the fruit, it is under the control of chromosome 2 [37]. Also, the difference in the thickness of the pericarp is related to

the ability of the genotype in the partition of assimilates leading to a thick or thin pericarp [38].

In addition, all fruits are pungent proving that all of genotypes pepper contain capsinoids, a group of closely related alkaloid found only in the genus *Capsicum* [39], especially capsaicin which is considered as the dominant pungency principle [40] which is localized in their placenta cellules [41,42]. In fact, the study of pungency in pepper was the focus of many researchers [43,44]. The determination of capsaicin content is considered as a criterion for genetic selection [45] and as commercial grade [46] due to its antioxidant activity [47] and its pharmaceutical properties [48].

5. Conclusion

From this study, morphological variations observed among the five Tunisian *Capsicum frutescens* accessions allow us to discover the biodiversity of local pepper landraces especially 'Souk Jedid' (Sj) accession which was significantly different from all other accession in all the characters studied. Thus, the five accessions could be divided into two groups, the first one includes 'Souk Jedid' (Sj) accession and the second one regroups the four other accession. After stability of the genotype, the performance of the five accessions must be studied to have more databases that could be interesting for breeding programs.

Table 2. Morphological characterization of five Tunisian chili pepper accession

	Tb	Sm	Kb	Az	Sj
Anthocyanin coloration of hypocotyl	9	9	9	9	9
Habit	3	3	3	3	1
length of stem	5	5	5	5	9
Shortened internode (in upper part)	1	1	1	1	9
Anthocyanin coloration of nodes	9	9	9	9	9
Intensity of nodes anthocyanin coloration	5	5	7	5	9
Hairiness of stem nodes	5	5	5	5	7
Length of blade (cm)	10.51 a	10.27 a	9.04 b	10.02 a	6.65 c
Width of blade (cm)	4.47 a	4.28 a	4.21 a	4.36 a	2.69 b
intensity of green color	5	5	5	5	7
shape	2	2	2	2	1
undulation of margin	1	5	1	1	1
blistering	5	3	5	5	5
glossiness	3	3	3	3	3
Anthocyanin coloration in anther	9	9	9	9	9
Corolla color	1	1	1	1	9
Peduncle attitude	3	2	2	3	1
Color before maturity	3	3	3	3	3
intensity of color	5	7	7	7	5
anthocyanin coloration	9	9	9	9	9
color in mature stage	3	3	3	3	3
intensity of color (at maturity)	7	7	7	7	7
Attitude	3	2	2	3	1
shape in longitudinal section	8	8	8	8	8
shape in cross section (at level of placenta)	3	3	3	3	3
sinuation of pericarp at basal part	9	5	3	3	5
sinuation of pericarp excluding basal part	1	1	1	1	1
texture of surface	2	1	1	1	1
glossiness	5	7	7	7	9
stalk cavity	1	1	1	1	1
shape of apex	1	1	1	1	1
number of locules	1	1	1	1	1
Calyx aspect	2	2	2	2	2
Pungency	9	9	9	9	9

Means in the same line followed by the same letter are not significantly different at 5% level according to Duncan test.

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