

FLORAL CHARACTERIZATION OF CAROB TREE (*CERATONIA SILIQUA* L.) FROM THE PROVINCE OF CHEFCHAOUEN (NW OF MOROCCO)

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Abstract. The survey of floral variability of carob tree (*Ceratonia siliqua* L.) from the Province of Chefchaouen (NW of Morocco) marks an unsteady floral mosaic. The determinate and the indeterminate inflorescences development is not controlled by environmental conditions in the study area. In carob tree, the indeterminate inflorescences rate is generally higher than the determinate inflorescences one. The particular inflorescence types have been observed. Inflorescence length and flowers number per inflorescence would not related to environmental conditions. Inflorescence length is superior in male trees when compared to female ones (5.71 cm vs. 4.45 cm). Flowers number per inflorescence in male trees is superior to the one in female trees (42 flowers vs. 30 flowers). In male carob tree, seven flower types have been distinguished differing by sepals and stamens numbers and filament length. In female carob tree, three flower types have been defined in terms of pistil form. The grafting of male or female trees in the domesticated areas affects the sex-ratio in this species. Pollen grains diameter and potential fertility show a high variability and could not be explained by the studied factors. The diameter of pollen grains is $28.3 \pm 2.43 \mu\text{m}$. The aborted pollen rate has been estimated to 15 %.

Key words: Floral variability, pollen, Sex-ratio, *Ceratonia siliqua* L., NW of Morocco.

Introduction

Carob tree (*Ceratonia siliqua* L.) represents the *Ceratonia* genera of the *Caesalpinoideae*, *Leguminosae*, *Angiospermae*, *Spermatophyta* [32]. It is dioecious, monoecious or rarely hermaphrodite [3, 6, 20, 25]. It grows in habitats of the low zone of Mediterranean vegetation with other species such *Pistacia lentiscus*, *Olea europaea* var. *sylvestris*, *Tetraclinis articulata*, *Juniperus phoenicea*, *Pinus halepensis* and *Quercus ilex*, forming the association of *Oleo-Ceratonion*, *Pistacio-Rhamnetalia* [1, 22, 33]. Carob culture is spread in the Mediterranean countries, Western Asia, Australia, South of Africa and USA [11].

In Morocco, it is spontaneous or cultivated in the thermo-Mediterranean and the meso-Mediterranean stages, corresponding to semi-arid and sub-humid bioclimates with minimal temperature over to 3°C and altitude up to 500 m and peculiarly up to 900-1600 m [12, 16, 18, 24, 33].

Many intra-specific varieties of carob tree have been reported in some Mediterranean countries in term of provenance and tree features [5, 6, 7, 11, 26]. Studies led on carob tree from Morocco distinguish several intra-specific types according to tree sex [22] or its domestication importance [13, 14]. Due to its elementary structure of flowers, carob

species is considered as an archaic taxon. Studies of floral development showed that flowers of carob tree have some irregular features contrary to most of *Leguminosae* [29]. The floral phenology in this species has been studied in Spain [25]. In Morocco, floral studies concerned only the dominant sex expression: male, female and hermaphrodite types [e.g. 22, 33]. Our study presents a more detailed floral description and an assessment of potential fertility of pollen in carob tree from the Province of Chefchaouen (NW of Morocco).

Material and methods

The study area is situated in the Province of Chefchaouen between 4°37'-5°54' W and 34°41'-35°27' N (figure 1). In order to establish the floral mosaic of carob tree, wild forest domain has been chosen because the sex-ratio is induced in the cultivated domain by grafting of spontaneous trees. From 75 carob trees (male, female and polygamous) occurring in 5 locations, we have sampled 2,250 flowers belonging to 750 inflorescences during flowering period in October 1999. Then they have been preserved in alcohol 95°. The macro-morphological characters of flowers have been studied by using light microscope. The percentage of flower types per sex is calculated on the basis of 10 inflorescences per tree. According to floral types previously identified, a complementary prospecting was carried out in 82 trees from the cultivated domain in order to evaluate the domestication effect on sex-ratio.

About 20 % of male flowers per inflorescence were crushed and mixed to assess pollen diameter and potential fertility, which were estimated on the basis of 30 measures and observations of 400 pollen grains per inflorescence in the same order. After differential staining, the normal pollen grains have red protoplasm (acid Fuschin) and green walls (green of malachite). The aborted pollen grains

haven't this coloration or with reduced protoplasm. Alexander's staining solution is composed of: ethanol 95% (10 ml), green of malachite (10 mg), distilled water (50 ml), glycerol (25 ml), phenol (5 g), chloral hydrate (5 g), acid Fuschin (50 mg), orange G (5 mg) and acetic acid (4 ml) (2). To avoid Cushing effect owed to pollen grains inflation, measures have been made simultaneously [8]. The data have been analysed by using Statistica 5.0 (Statsoft Inc. 1995) software.

Results

Floral mosaic

Carob species presents an extended flowering period. The inflorescence development corresponds to 4-6 weeks in male trees and c. 8 weeks in female trees. Two inflorescence types were identified: determinate inflorescence having a terminal flower and indeterminate inflorescence without terminal flower. The growth type of carob inflorescence in the wild forest domain could not identify differential groupings in terms of provenance (table 1). We have identified 4 inflorescence types: male, female, polygamous and hermaphrodite. The indeterminate inflorescences characterize more female trees (91.7 % vs. 88.0 %); while the determinate inflorescences distinguish more male trees (12 % vs. 8.33 %) (table 2). But the rate of determinate inflorescences is generally low not exceeding 10.2 ± 18.7 % of sample strength. Moreover, inflorescences are isolated and particularly grouped in whorls or ramified (figure 2). Moreover, Tucker (1992) has reported some similar characteristics to *Gleditsia* as simple, branched, determinate or indeterminate inflorescence and functional unisexuality. Leaflets alternate sometimes with flowers at the lower part of the inflorescence axis (figure 3).

Environmental conditions would not explain the very highly significant differences of quantitative parameter

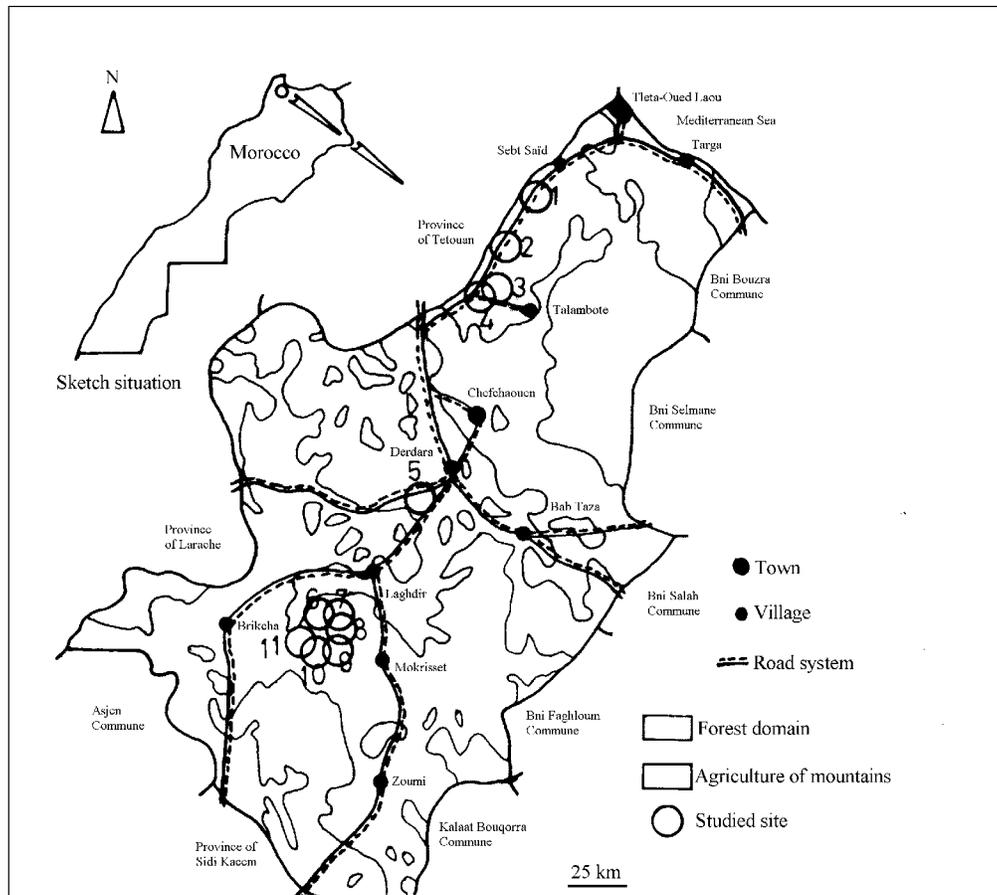


Figure 1. Situation of the survey area [9, Road map of Morocco 1/400000].



Figure 2. Ramified inflorescence (x1.7)

(table 3). The analysis of variance between trees from the same location shows very highly significant differences too (N=150).

The average of inflorescence length varies from 4.37 cm to 6.12 cm and the flowers number per inflorescence reaches 32-40 flowers i.e. a floral density of 6-8 flowers/cm of inflorescence axis reflecting a high faculty of flower production in this species.



Figure 3 Leaflet development on inflorescence axis (x1.4)

Table 1. Inflorescence types frequency respect to studied locations.

Location (figure 1)	Indeterminate Inflorescences (%)		Determinate Inflorescences (%)		SD
	mean		mean		
Oued Takikis	86.0		14.0		18.4
Oued Amaghous	87.3		12.7		17.9
Talambote	99.3		0.67		2.58
Ighoujja	91.3		8.67		13.0
S.M. Ben Saada	80.7		19.3		28.1
Total mean	88.9		11.1		18.6
Maximum	100				
Minimum	0.00				
F ratio	2.226				
P	0.075				
Location effect	NS				

N=75. NS, insignificant.

Table 2. Inflorescence types frequency respect to tree sex.

Sex	Indeterminate inflorescences (%)		Determinate inflorescences (%)		SD
	mean		mean		
Male	88.0		12.0		21.2
Female	91.7		8.33		16.0
Total mean	89.8		10.2		18.7
Maximum	100				
Minimum	0.00				
F ratio	0.571				
P	0.453				
Sex effect	NS				

N=60. NS, insignificant.

Table 3. Inflorescence quantitative parameters respect to studied locations.

Location (figure 1)	Inflorescence length (cm)	Flowers number / inflorescence	Flowers number / cm
Oued Takikis	5.55±2.09 a	35.3±14.3 ac	6.77±2.61 ab
Oued Amaghous	5.03±2.21 b	39.7±17.8 d	8.28±2.91
Talambote	4.37±1.52	31.7±11.9 b	7.42±1.79
Ighoujja	5.34±1.80 ab	34.9±12.0 abe	6.79±2.13 ac
S. M. Ben Saada	6.12±2.65	37.8±15.2 cde	6.59±2.25 bc
F ratio	14.44	6.534	12.96
P	0.000	0.000	0.000
Location effect	***	***	***
Total mean	5.28±2.16	35.9±14.6	7.17±2.44
Maximum	13.9	87.0	16.5
Minimum	1.10	7.00	2.29

Values followed by the same letter are not significantly different at 5 %, N=750.

The sex effect showed very highly significant differences for inflorescence length and flowers number per inflorescence. While floral density has not showed significant differences (table 4). Inflorescence length and flowers number per inflorescence are superior in male trees when compared to female ones (5.71 cm vs. 4.45 cm and 42 flowers vs. 30 flowers in the same order), showing that the male

carob trees produce more flowers than the female ones.

Observations on the floral primordia show that they are bisexual and only one sexual organ type becomes functional. During the floral ontogeny, we have observed that in female flowers, the pistil continues its development while stamens remain blacked and without filaments elongation nor production of

pollen grains. In male flowers, the stamens are developed and pistil remains atrophied without elongation nor ovules production. The suppression of sexual organ development occurs in early stage of floral organogeny.

The provenance effect marks weakly to very highly significant differences (table 5) and the intra-location or tree effect is very highly significant (N=80) displaying an unsteady floral mosaic in this species. The floral variability in male trees is especially expressed in this survey by sepals and stamens numbers and filaments length. Thus, we distinguished seven male flower types (table 5). The (5 S + 5 E) flower type is more represented (71.6 %), followed mainly by (5 S + 6 E) and (6 S + 5-6 E with at least 2 knitted stamens in filaments and/or anthers) flowers types (table 5, figure 4).



Figure 4. Stamens partially knitted (x5)

In terminal flower of male inflorescences, having sometimes a spatulate nectar disk, sepals and stamens numbers vary respectively from 5 to 8 S

and from 5 to 14 E of which at least two are knitted (e.g. figure 5). The sixth sepal is in an external position and identical or smaller as to the other ones. In male flowers, the pistil is rudimentary and considered no functional. The filament length can vary within the same flower. The ANOVA of stamen filament length highlighted very highly significant differences between trees from the same location (N=240) and within the same tree (N=30) (table 6) marking a polymorphism of this parameter.

Respect to female flowers, prospecting of different locations has not showed any significant differences and form a homogeneous group. We have identified three female flower types having 5 or 6 sepals and differing by the pistil form: curved (35 %), moderately curved (34.8 %) and straight (30.2 %) pistils (table 7). External factors as climate and/or endogenous features of plant could determinate pistil curvature.

Moreover, we have observed in female flowers bract in flower axil, grouping of two or more flowers with differential development on the same point of inflorescence axis, and female flower with no functional stamen. The terminal flower in female inflorescences, resembling sometimes to spatula, present 4, 5, 8 or 10 sepals and 1, 2 or 4 independent pistils or partially knitted (e.g. figures 6 & 7).

The hermaphrodite flowers identified in polygamous and hermaphrodite inflorescences have

Table 4. Inflorescence quantitative parameters respect to tree sex.

Sex	Inflorescence length (cm)	Flowers number / inflorescence	Flowers number / cm
Male	5.71±2.14	42.2±15.4	7.67±2.08
Female	4.45±1.79	30.3±11.7	7.31±2.78
F ratio	61.05	113.4	3.251
P	0.000	0.000	0.072
Sex effect	***	***	NS
Total mean	5.08±2.07	36.2±14.9	7.49±2.46
Maximum	13.9	87.0	16.5
Minimum	1.10	7.00	2.34

N=600.

5 sepals, 5-6 stamens and 1 pistil. Filaments and anthers persist after pollination until the mature fruits stage.

Sex-ratio

The carob sex-ratio has been estimated both in wild and cultivated domains (figure 8). Grafting of spontaneous male and female trees, having a weak carob yield, induces strongly sex-ratio. Male trees are generally preserved for pollination of female ones or in field hedges. In terms of sample strength, sex-ratio is superior in strict wild locations (Oued Takikis and S.M. Ben Saada) and domesticated locations (Alma, Jnane Biada), lower or equal to zero when grafting is intensively practiced in the other locations.

In the Talambote and Alma locations (figure 1), we have identified

carob trees with male and female flowers on different branches like a monoecious type. But, more observations and interviews with autochthonous farmers confirm that these trees have been partially grafted during the earlier stage. Prior to sex determination of carob tree, it is necessary to collect information about its domestication (upkeep, grafting...). The trimonoecious tree type is very scarce not exceeding 7 % in this study. In prospecting area, no hermaphrodite tree type was observed.

Pollen diameter and potential fertility

The average pollen grain diameter corresponds to the mean of its polar and equatorial axis. The results consigned in the table 8 show that location effect marks very highly significant differences defining

Table 5. Flower types frequency (%) in male carob tree respect to studied locations.

Location (figure 1)	4S+4E	5S+5E	5S+6E	5S+7E
Oued Takikis	0.56±2.57 a	65.8±39.3 a	1.36±2.97 ab	0.00±0.00 abd
Oued Amaghous	0.00±0.00 bc	92.6±5.65	1.02±4.07 a	0.00±0.00 ace
Talambote	0.00±0.00 d	64.9±43.6 ab	5.88±7.27 b	0.00±0.00 bcf
Ighoujja	0.25±1.07 abe	80.2±13.5	11.9±13.5	0.10±0.55 def
S.M. Ben Saada	0.05±0.25 cde	61.5±28.1 b	20.1±15.7	2.76±3.89
F ratio	3.597	17.80	41.71	36.85
P	0.006	0.000	0.000	0.000
Location effect	**	***	***	***
Total mean	0.12±0.88	71.6±30.0	11.3±13.9	0.95±2.57
Maximum	13.9	100	50.0	17.4
Minimum	0.00	0.00	0.00	0.00

	6S+5E	6S+6E	6S+7E
	17.5±23.7 a	14.9±16.1	0.00±0.00 abce
	0.00±0.00 b	6.34±5.36 abd	0.00±0.00 ad
	24.5±38.6 a	4.28±9.26 ace	0.48±2.16 bf
	0.00±0.00 b	7.62±13.1 bcf	0.00±0.00 cd
	7.08±21.4	8.04±21.6 def	0.46±1.94 ef
	18.96	2.858	2.926
	0.000	0.023	0.020
	***	*	*
	8.43±24.3	7.40±15.4	0.25±1.47
	93.3	100	11.5
	0.00	0.00	0.00

Values followed by the same letter are not significantly different at 5 %, N=150.



Figure 5. Terminal male flower with short filaments and 11 stamens (x5)

Table 6. Stamen filament length in male flowers respect to studied locations.

Location (figure 1)	Stamen filaments length in male flowers (cm)
Oued Takikis	0.47±0.14 a
Oued Amaghous	0.30±0.10 b
Talambote	0.35±0.12 c
Ighoujja	0.46±0.16 a
S. M. Ben Saada	0.33±0.11 bc
F ratio	30.55
P	0.000
Location effect	***
Total mean	0.38±0.14
Maximum	0.8
Minimum	0.1

Values followed by the same letter are not significantly different at 5 %, N=450.

Table 7. Female flower types respect to studied locations.

Location (figure 1)	Flowers with straight pistil (%)	Flowers with moderate curved pistil (%)	Flowers with curved pistil (%)
Oued Takikis	34.0±37.2	27.0±31.6	39.0±41.5
Oued Amaghous	28.0±33.9	30.0±29.4	42.0±41.6
Talambote	38.0±30.0	47.0±24.1	15.0±21.7
Ighoujja	35.0±45.8	22.0±22.0	43.0±34.7
Sidi Mohamed Ben Saada	16.0±17.1	48.0±31.2	36.0±38.4
F ratio	0.654	1.827	1.005
P	0.627	0.140	0.415
Location effect	NS	NS	NS
Total mean	30.2±33.7	34.8±28.9	35.0±36.3
Maximum	100	90.0	100
Minimum	0.00	0.00	0.00

N=50. NS, insignificant.

**Figure 6.** Female flower with spatula form and two pistils (x3.3)**Figure 7.** Terminal female flower with three pistils partially knitted (x3.3)

four locations groups. The pollen diameter varies from 27.6 μm to 29.1 μm with a total mean of 28.3±2.43 μm . This variability would not be related to environmental circumstances. Analysis of variance of tree effect or intra-location (N=2,400) and of inflorescence effect or

intra-tree (N=300) underlined a conspicuous intra-individual variability of pollen diameter.

The Alexander's test revealed that all trees have pollen grains fertile with red protoplasm and green walls in Oued Takikis, Oued Amaghous and Talambote.

The aborted pollen is considered sterile and presents a rate from 2 to 15 %. However, we obtained respectively 67 % and 33 % of potentially fertile and sterile pollen rates in Ighoujja (table 8). The non aborted pollen could be physiologically no functional because of the overestimation of pollen fertility by Alexander's staining. Furthermore, analysis of variance of trees or intra-location effect (N=80) revealed very highly significant differences.

Discussion

From a floral viewpoint, *Ceratonia siliqua* presents a strong plasticity. Hermaphrodite inflorescences are very scarce respect to male, female and polygamous ones. The same observation was mentioned in carob tree from Spain and Portugal [3]. The indeterminate inflorescences characterize more female trees and the determinate ones mark more male trees. The low rate of determinate inflorescences is a

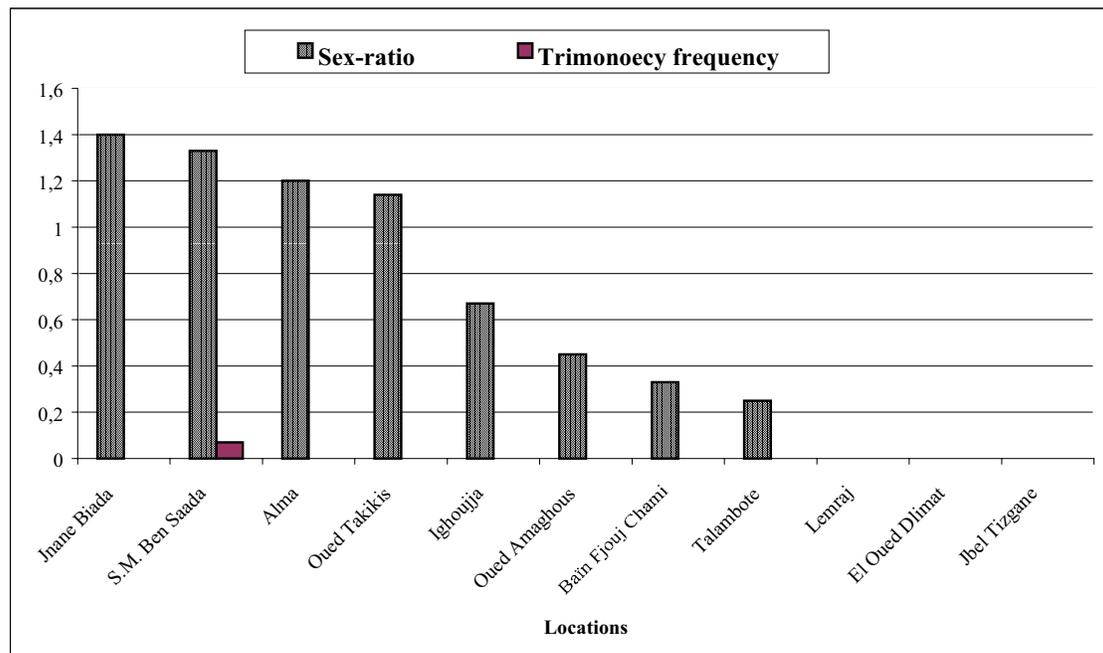


Figure 8. Sex-ratio and trimonoecy (%) in carob tree from wild and domesticated locations.

Table 8. Pollen diameter and potential fertility respect to studied locations.

Location (figure 1)	Pollen diameter (μm)	Pollen potential fertility (%)
Oued Takikis	29.1 \pm 2.50	95.5 \pm 3.79 ab
Oued Amaghous	28.2 \pm 2.27 a	90.1 \pm 5.55 ac
Talambote	28.3 \pm 2.25	98.0 \pm 2.55 b
Ighoujja	28.1 \pm 2.17 a	67.2 \pm 13.1
S. M. Ben Saada	27.6 \pm 2.71	85.2 \pm 21.2 c
F ratio	43.87	101.2
P	0.000	0.000
Location effect	***	***
Total mean	28.3 \pm 2.43	84.6 \pm 17.4
Maximum	40.0	100
Minimum	21.0	0.00
N	4 500	150

Values followed by the same letter are not significantly different at 5 %.

characteristic of *Leguminosae* having mainly the indeterminate inflorescences [29].

Inflorescence length and flowers number per inflorescence would not depend on environmental conditions. Other authors have reported that flowers number per inflorescence is high in male carob tree (43.5 vs. 40) and low in the female one (17 vs. 30) [25]. Thus, male carob tree emphasizes particularly a high faculty of flowers production increasing pollination and fertilization of female flowers. Carob species presents also a long flowering period (autumn - winter) like many tropical plants [11, 33].

In this study we observed like other authors [e.g. 16, 20] three flower types in this species: male, female and hermaphrodite. Suppression of sexual organ development occurs in an earlier stage of floral organogeny analogously to other *Leguminosae* [31]. Moreover, floral unisexuality could be expressed by different mechanisms in any stage of floral ontogeny [30]. According to other works, all flowers are originally bisexual and without petals becoming functionally unisexual because of male or female organs suppression [28]. In other *Leguminosae* such as *Neptunia pubescens*, *Bauhinia malabarica* and *B. divaricata*, all flowers are bisexual in the early stage and become unisexual by a subsequent suppression of one of the two organs [27, in 28].

The floral mosaic of carob tree is unsteady, generating several types of male flowers in terms of sepals and stamens numbers, and female flowers differing by pistil form. In agreement with our results, authors have underlined that male flowers have 5-6 sepals and 5-7 functional stamens [17]. The filament length defines two male between polar diameter of carob pollen grain ($23.9 \pm 1.83 \mu\text{m}$) and equatorial one ($23.2 \pm 1.86 \mu\text{m}$) [19]. In *Ceratonia siliqua*, aborted pollens rate reaches 36 % vs. 15% in this study [28]. The male sterility could be explained by meiotic anomalies (male

flowers types (with short or long filaments) agreeing with Tucker's quote [28]. Authors use filament types to characterize male inflorescences (inflorescences with short or long stamen filaments) in addition to female, hermaphrodite or polygamous inflorescences [in 3].

The hermaphrodite carob tree has not been identified in this survey and the triecy characterizes apparently this species. It is generally dioecious or monoecious and rarely hermaphrodite [3, 6, 20, 23, 24, 25]. The identified carob trees during this prospecting are whether dioecious (male and female flowers on different trees), or polygamous or trimonoecious (male, female and hermaphrodite flowers on the same tree) [10]. The floral unisexuality is considered like a developed character from a bisexual ancestral stage and presents a reversible change capacity in relation to floral bisexuality [28].

The grafting of the male and female trees in the domesticated domain influence the carob sex-ratio since authors have quoted that It is equal to one [3], displaying a high variation of this ratio in terms of culture and distribution areas. It could be concluded that carob tree from Chefchaouen Province is dioecious and scarcely trimonoecious (uni- and heterosexual flowers on the same tree or even the same inflorescence). However, "artificial" monoecious trees (unisexual flowers on different branches and/or bushes of the same tree) are related to grafting and upkeep factors.

The studied factors could not explain the high variability of carob pollen diameter and fertility. The average diameter of pollen grain is $28.3 \pm 2.43 \mu\text{m}$ conferring with values underlined by other authors [17]. It has emphasized a similarity (gametogenesis blockage) or pollen grains abortion due probably to particular energy demand of plant. In allogamous species, male fertility and pollen production depend on environmental factors such as temperature and light, which induce the

total quantity of available resources for plant metabolism [4, 15].

Our data show that variation of diameter and fertility of carob pollen could be related to an unsteady reproduction strategy in this species. Carob tree presents many features favouring pollination conditions such as high production of seeing flowers with strong odour, sticky and sweet nectar disk. Pollination agents are generally flies, bees, wasps, ants and moths [17, 21].

In the survey zone, carob species is dioecious and very rarely trimonoecious. It develops male, female, polygamous or hermaphrodite inflorescences according to tree sex. The floral mosaic in this species includes several types of male and female flowers. Alexander's test shows that potential pollen fertility presents a high variability. Complementary studies particularly of pollen grain germination would be necessary to evaluate actual pollen viability.

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