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Study of gender effect on leaf morphology of *Ceratonia siliqua* L. in Cheurfa stand (Tigzirt, Tizi Ouzou)

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Dedication

This dissertation is dedicated to the sake of ***Allah*** my creator, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this work. I also dedicate this work to my parents ***Saliha*** and ***Rabah***, my brothers ***Abdelhak*** and ***Noureddine*** for their love, support and confidence during all my academic steps and difficult moments. I would never have got to the end without you thank you very much.

I dedicate this dissertation and give special thanks to my dear friends' ***Saliha Yassmin*** and ***Houria*** for their encouragement and friendships.

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Abstract

In this study we analyzed the effect of gender on leaf morphology of *Ceratonia siliqua* L. in Cheurfa strand (Tigzirt, Tizi-ouzou). We assessed various morphological characters of male and female leaves (leaf length, leaf width, petiole length, rachis length, number of leaflets, basal leaflet length, basal leaflet width, terminal leaflet length, terminal leaflet width, specific leaflet weight) and micro-morphological trait (number of stomata per mm square). The statistical analysis (ANOVA) showed significant difference between males and females in the number of stomata. Female trees have higher number of stomata than male trees. However we didn't find significant differences in the other leaf traits.

Keywords: Gender, *Ceratonia siliqua* L., leaf morphology, Cheurfa (Tigzirt, Tizi-ouzou), leaf micro-morphology.

Résumé

Dans cette étude, nous avons analysé l'effet du genre sur la morphologie foliaire chez Ceratonia siliqua L. dans la station de Cheurfa (Tigzirt, Tizi-Ouzou). Nous avons évalué différents caractères morphologiques foliaire mâle et femelles (longueur de la feuille, largeur de la feuille, longueur du pétiole, longueur du rachis, nomber de folioles, longueur de foliole basale, largeur de foliole basale, longueur de foliole terminale, largeur de foliole terminale, poids spécifique) et trait micro-morphologique (nombre de stomates par mm carré). L'analyse statistique (ANOVA) a montré une différence significative entre les mâles et les femelles dans le nombre de stomates. Les arbres femelles ont un nombre de stomates plus élevé que les arbres mâle. Cependant, nous n'avons pas trouvé de différences significatives dans les autres traits de feuille.

Mots clés : genre, *Ceratonia siliqua* L., morphologie follaire, Cheurfa strand (Tigzirt, Tizi-Ouzou), micro-morphologie foliaire.

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List of abbreviations

Ll: Leaf length

Lw: Leaf width

Pl: Petiole length

Rl: Rachis length

Lfn: Leaflets number

Blfl: Basal leaflet length

Blfl: Basal leaflet width

Tlfl: Terminal leaflet length

Tlfw: Terminal leaflet width

Slfwe: specific leaflet weight

Sq: square

SE: standard error

SD: standard deviation

Ns: not significant

Fig: figure

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Introduction

Introduction

Carob tree is a long-lived evergreen tree (*Ceratonia siliqua* L.) that has been widely cultivated around the world over the years and described as a plant with a large adaptability in the Mediterranean area. It grows well in warm temperate and subtropical areas, and tolerates hot and humid coast areas. As xerophytic (drought-resistant), this specie has developed special mechanism to survive in the Mediterranean conditions, and its introduction in semi-arid lands may help to prevent the disruption of the equilibrium of those fragile ecosystems (Batlle & Tous, 1997).

In Algeria, the carob tree is found everywhere in the north of the country, and even in north of sahara (desert) in altitudes of less than 1700m (Arbane, 2015). It thrives together with a number of other species such *Pistacia lentiscus* L. and *Olea europaea* L. *var. sylvestris*, forms one of the most characteristic associations of the lowest zone of the Mediterranean vegetation and thus is considered to be a climax community (*Oleo-Ceratonion*) (Batlle & Tous, 1997).

The carob tree plays an important socio-economic and ecological role in the agrosilvopastoral and animal feed system (Moreno et al, 2014). The leaves of this tree are frequently used to cure various diseases such as diarrhoeal, diabetes, obesity, cancers (breast, cervical, and colon cancers), liver diseases (Ghanami & Belarbi, 2021).

This species (*Ceratonia siliqua*) is dioecious with some hermaphroditic forms, which means each tree bears male or female or hermaphrodite flowers (Batlle & Tous, 1997). In addition to having different roles in reproduction. Moreover the differences in morphological and physiological traits have been documented between gender of dioecious plants by many researchers (Iszkuło et al., 2000; Hultine et al, 2007; XU et al., 2008 among others). However, there is few published work on morphological features of male and female *Ceratonia siliqua* trees if we except Gharnit et al., 2005, therefore, the present study was conducted with the aim to analyze leaf morphological traits between female and male of *Ceratonia siliqua* trees.

Chapter 1:

Literature Review

I. The carob tree

1. Botanical systematics of carob

The scientific name of the carob tree (*Ceratonia siliqua L.)* derives from the Greek "keras", horn, and Latin "siliqua", alluding to the hardness and shape of the pod. The common name originates from the Hebrew kharuv, from which other vernacular names are derive (St. John's bread, locust bean, carob bean...) (Batlle & Tous, 1997).

The carob tree (*Ceratonia siliqua L.)* belongs to family Fabaceae, subfamily Caesalpinoideae, tribus Caesalpinieae which includes the genus Ceratonia that consist of two plants species: carob *(C. siliqua*) and *Ceratonia Oreothauma* (located in Arabia and the republic of Somalia) (Srečec et al., 2020).

According to Srečec et al., (2020), the detailed systematics of carob is shown in Table 1.

Table 1: Botanical systematics of carob

Taxonomic unit	**Name**
Class	*Magnoliopsida*
Subclass	*Rosidae*
Suborder	*Fabanae*
Order	*Fabales*
Family	*Fabaceae*
Subfamily	*Caesalpinioideae*
Tribus	*Caesalpinieae*
Genus	*Ceratonia*
Species	*Ceratonia siliqua* L.

2. Description

The carob is a long-lived evergreen (over 150 years) that grows to a height of 4 to 15m (Fig. 1), it has a large semispherical crown with one or more trunks and sturdy branches (Albanell, 1990).

2.1 Leaves

Leaves are 10 to 20 cm in length, alternate, pinnate, with or without a terminal leaflet (Fig. 1), Leaflets are between 3 and 7 cm long and arranged on opposite sides of the stalk, there are 4 to 10 pairs of leaflets, oval, rounded at the tip, dark green on top and matt green on their back.

The leaves are sclerophyllous and characterized by a thick waxy coating that prevents excessive moisture loss in semi-arid climates. In July of alternating years the leaves are shed and replaced with new (Batlle & Tous, 1997).

Figure 1: A large carob tree (left) and Leaves on the carob tree (right), Bouzeguene (Sahel), May 2021.

2.2 Flowers

Flowers are small and numerous, between 6 to 12 mm long, arranged in spicate inflorescences that develop in the axil of the leaves or on woody branches or on the trunk, and being separated by sexes in different trees (Batlle & Tous, 1997). The flowers of the male tree are stamen clusters with pollen, producing a very strong odor (Fig. 2a), while the female produces small, yellow aromatic flowers (pistils), grouped in clusters (Fig. 2b). Hermaphrodite flowers are a combination of both type containing a pistils and a stamens (Fig. 2c).

Figure 2: Flowers of carob tree: a) male flowers, b) female flowers, c) hermaphrodite flowers (male flowers and pods), Tigzirt (Thala testane), Photos by Nadia Abdelkrim.

2.3 Fruit

The fruit of the carob is a pod that is elongated, compact, straight, curved, or twisted shape depending on the specific variety (Zografakis & Dasenakis, 2000 *in* Iipumbu 2008). The pod reaches a length of 25 cm, a width of 4 cm, a weight between 5 and 30 g and a thickness of 1.3 cm varies somewhat between varieties (Marakis, 1992 *in* Iipumbu 2008). It is green when immature but then maturing to dark brown color and indehiscent (Fig. 3a,b). The pod is

separated inside by pulpy partitions (an outer leathery layer "pericarp", and an inner softer layer "mesocarp") and contains 5 to 18 seeds arranged in transverse position separated by the mesocarp (Batlle & Tous, 1997) (Fig. 3c).

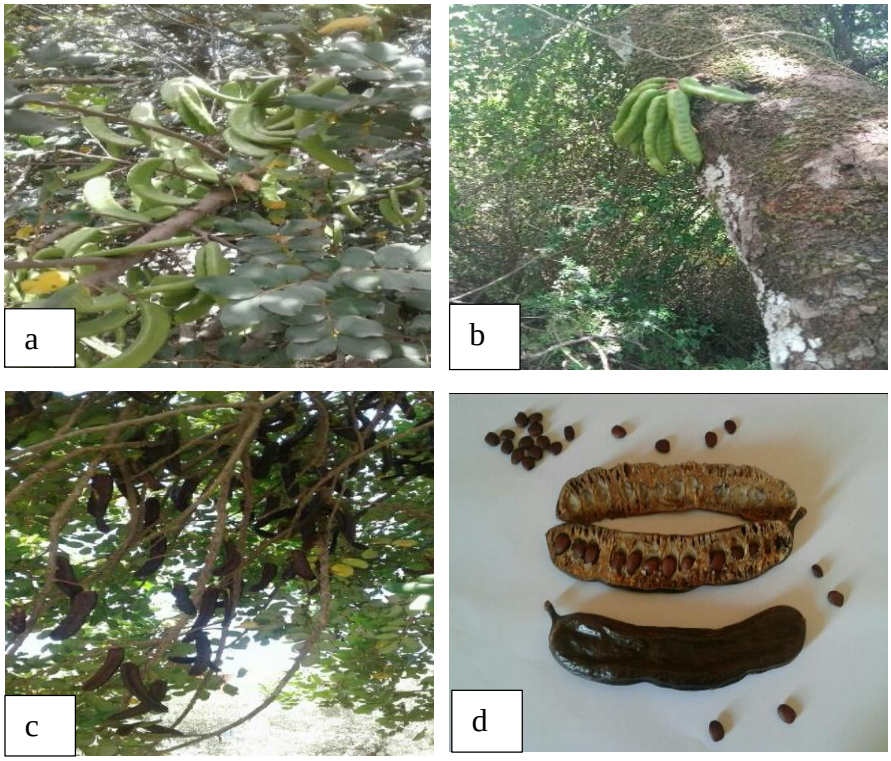


Figure 3: Carob pods: a,b) green pods, c) ripe pods, d) the arrangement of seeds in the pods, Bouzguene (Sahel), between May and July2021.

2.4 Seeds

Seeds are numerous, compressed, slightly oblong and are about 8 to 10 mm, 7 to 8 mm wide and 3 to 5 mm thick. Each seed is covered by testa which is hard, smooth, shiny brown and represent 30 to 33% of the seeds weight (Batlle & Tous, 1997) (Fig. 4).

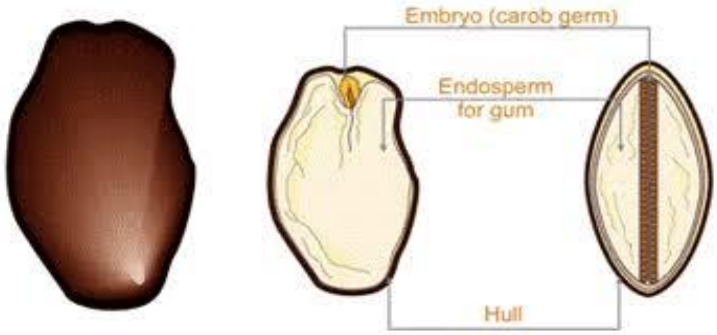


Figure 4: carob seed structure (Anonymous, 2009b *in* M. Smith 2009).

2.5 Roots

The carob tree has an extensive root system with a deep taproot (up to 20m) and lateral roots (Fig. 5), this root system allows the absorption of water and minerals from a large area of land and anchors the tree to the ground (Albanell, 1990). However, it is unable to fix nitrogen (Batlle & Tous, 1997; Albanell, 1990)

Figure 5: Extensive root system in Tigzirt (Thala testane), Photo by Nadia Abdelkrim.

2.6 Trunk and branches

The trunk is thick (Fig. 6), sometimes twisted with circumference of 3 to 7m (Albanell, 1990). It has a smooth gray bark at young age (El Kahkahi et al., 2016), brown and rough when it mature (Batlle & Tous, 1997; El Kahkahi et al., 2016), and grayish-brown according to (Albanell, 1990).

The branches have different characteristics (Albanell, 1990):

- The main branches (older): are generally thick, twisted and with a tendency to horizontality (Fig. 7a). Their main role is to serve other branches and even sometimes can be productive.
- The secondary branches (medium size): arising from the main branches, they are generally productive (Fig. 7b).
- The young branches or twigs (smaller): are located in outer part of the crown or growth zone, they are flexible and have a smooth bark covered with lenticels that allow gas exchange with atmosphere.

Figure 6: Different shapes of carob trunk in Bouzeguene (Sahel), May2021.

Figure 6: Carob branches: a) main branches, b) secondary branches in Bouzeguene (Sahel), May2021.

3. Reproductive biology

The carob tree is dioecious, sometimes hermaphrodite and rarely monoecious (Batlle & Tous, 1997; Retana et al., 1994). It is considered the only Mediterranean tree with a main flowering season similar to that tropical plants (September to November). However, the time and length of the flowering period depends on local climatic conditions, geographical location, sex and variety, among other factors (Batlle & Tous, 1997; Albanell, 1990).

The flowering period differs in male and female trees, namely male trees bloom earlier, and this difference can even be 2.3 weeks (Srečec et al., 2020). Moreover, flowering period in male trees is shorter than in hermaphrodite and female trees (Retana et al., 1994).

Flower numbers and density in female inflorescences are lower than in male and hermaphrodite inflorescences (Retana et al., 1994). These flowers of three sexes secrete nectar and attract large numbers of insects.

The pollination is carried out by wind and insects (Batlle & Tous, 1997; Albanell, 1990), only female and hermaphrodite flowers which are pollinated will develop into carob pods.

The development of fruit occurs slowly during the late winter and spring. The pod requires 10 months to mature (Batlle & Tous, 1997; El Kahkah et al., 2016). It elongates rapidly between February and late May/early June when it reaches its maximum size. During June its color change to a chocolate-brown and it is fully matures towards the end of August (Davies, 1970).

The carob tree is characterized by alternate bearing, this behavior has linked to the presence of fruit remaining on the tree, endogenous factors, climatic conditions such as scarce and variable rainfall, frost, fog, lack of pollination, damage of the inflorescence during harvesting (knocking down of the fruit), and deficiencies in orchard management (fertilization, weed control, irrigation, pruning, etc.) (Batlle & Tous, 1997; Curtis & Race, 1998).

4. Origin and geographical distribution

4.1 Origin

The carob tree (*Ceratonia siliqua)* has been grown and cultivated since ancient times, even though the exact origins of carob tree are unknown. Probably native to the eastern Mediterranean region, mainly to Middle-East countries (Batlle & Tous, 1997).

4.2 Geographical distribution

4.2.1 In the world

The species (*Ceratonia siliqua*) may be found in many parts of the world, especially ones with a climate similar to the Mediterranean climate, including South Africa, USA, Australia and Chili. However, the species is widely distributed in the Mediterranean countries such as Morocco, Algeria, Tunisia, Libya, Egypt, Israel, Lebanon, Syria, Turkey, Greece, Italy, France, Spain and Portugal (Batlle & Tous, 1997) (Fig. 8).

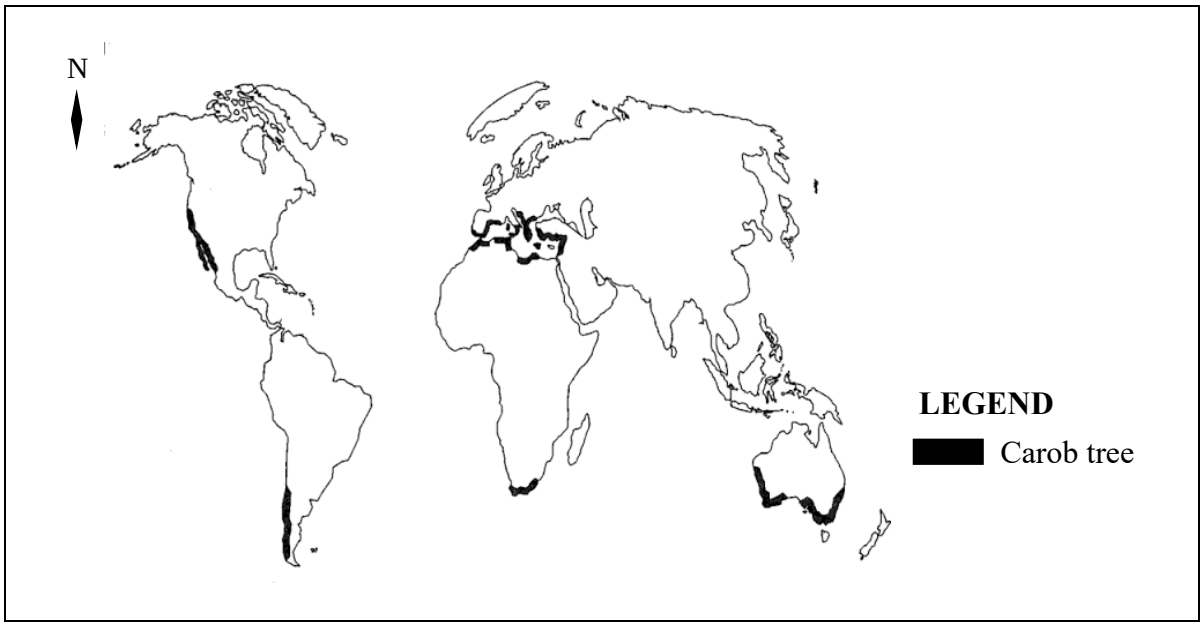


Figure 8: World distribution of the carob tree (Batlle & Tous, 1997).

4.2.2. In Algeria

In Algeria the carob tree is found everywhere in the north of the country, and even north of sahara in altitudes of less than 1700m (Arbane, 2015). However it is widely cultived in the north and especially in following wilayas (provinces): Bejaia, Tipaza, Blida, Boumerdes, Bouira, Mila, Tlemcen, Bordj Bou Arreridj, Ain-Defla, Mascara and Tizi-ouzou (DSA de Tlemcen, 2009 *in* Mahdad & Gaouar, 2016).

5. Ecology

The carob is a Mediterranean thermophilous tree, it is thriving in warm temperate and subtropical areas, and tolerates hot and humid coastal areas. As a xerophytic (drought-resistant species), carob is well developed to the condition of the Mediterranean region. It is also thrives together with a number of other species such *Pistacia lentiscus L.* and *Olea europaea L. var. sylvestri*s, forming the association of Oleo-Ceratonion (Batlle & Tous, 1997).

5.1 Climate requirements

The carob does best in a Mediterranean-type climate with cool, not cold, winters, mild to warm springs, and warm to hot dry summers. It can withstand temperatures of 40°C for long periods of time. However, it is not able to withstand temperatures below -7°C and receives significant damage at temperatures of -4°C with different varieties being able to withstand different temperature extremes (Batlle & Tous, 1997).

5.2 Soil requirements

The carob tree grows in many different soil types: sandy, rocky, clay loams, limestone, and alkaline or moderately acid soils (Albanell, 1990; Curtis & Race 1998), but it prefers sandy well-drained loams but calcareous soils with high lime content are also suitable and is intolerant of waterlogging and saline soils and schistose crusts (Batlle & Tous, 1997; El Kahkahi et al., 2016).

5.3 Water requirements

Adult carobs can survive in areas with a rainfall of 250 mm/year as they tolerate to drought conditions, but to produce a commercial crop, they need 500 to 550 millimeters of rainfall per year. Whatever 350 mm of annual rainfall are considered enough for fruit set (Batlle & Tous, 1997; Curtis & Race 1998).

6. Propagation techniques

Carob tree can be propagated by seeds, cuttings, grafting, or micro-propagation (El Kahkahi et al., 2016), and has been described by many researchers as one of the most difficult species to root (Lee et al., 1977; Hartman & Kester, 1983 *in* Batlle & Tous, 1997).

6.1 Direct seeding

Carobs seeds or seedlings are planted in the autumn or early spring (Curtis & Race, 1998). There are two ways to plant the seeds:

- Pre-soaked in water and planted to a depth of 5 cm.
- Pre-germinated and planted to a depth of 2.5 cm where supplementary irrigation is available.

6.2 Cuttings

The cuttings, in March or April, require a rich substrate, hormonal treatment and warm conditions (Batlle & Tous, 1997; Zaen El Deen, 2014). Its success depends on the genotype and type of shoot (age and position) (Batlle & Tous, 1997). Cuttings involve removing portions of twigs under specific conditions allowing them to form a bark ridge and you can make root (El Kahkahi et al., 2016).

6.3 Grafting

The aim of grafting is to obtain suitable varieties and to increase productivity per tree. It involves grafting male branch onto a female tree (El Kahkahi et al., 2016; Curtis & Race, 1998).

6.4 Micropropagation

Micropropagation is considered a technique for the preservation of plant genetic material, therefore the use of this technique can be the solution to solve propagation problems in Carob and to extend its propagation (Zaen El Deen, 2014).This technique using seedlings and adult plants (Batlle & Tous, 1997), as well as various explants: nodes taken from germination plantlets (El Kahkahi et al., 2016), axillary buds (Romano et al., 2002).

7. Uses

The carob tree has been cultivated for a long time for various uses. It is an ecologically important tree, socio-economic, industrial and ornamental indisputable (El Kahkahi et al., 2016). The entire plant (leaves, flowers, fruits, seeds, wood, bark and root) is in high demand and have heavily exploited.

7.1 Tree

The carob tree is used as an ornamental plant along roadsides and in orchards. It is used as windbreaks around orchards because of its dense foliage, it is also helpful buffers against noise from factories, roads and railways. It may also be helpful in degraded areas threatened by soil erosion and desertification (Batlle & Tous, 1997).

7.2 Leaves

The leaves are frequently used to cure various diseases such as nephyropathy, liver diseases, diabetes, obesity, inflammatory bowel disease, diarrhoeal, new castle disease, Parkinson's diseases and cancers (breast, cervical, and colon cancers). The leaves are also used for their antioxidant and antimicrobial activities (Ghanami & Belarbi, 2021).

7.3 Pods

Carob pods have traditionally been used not only in feeding ruminant animals or non-ruminant, but also in human food. The pods are used after crushing to separate seed and pulp (Batlle and Tous, 1997).

7.3.1 Pulp

The pulp is ground into a fine powder and used as cacao substitute when toasted to make chocolate and cakes, ice cream, alcohol (Batlle and Tous, 1997). In traditional medicine, the pulp is used against diarrhea and for the treatment of certain diseases such as gastritis, enteritis, tonsillitis, colds, cancer (El Kahkahi et al., 2016).

7.3.2 Seeds

The seeds are processed into an edible gum is commonly known as carob bean gum or locust bean gum (Batlle & Tous, 1997). This gum obtained by grinding up the endosperm of carob seeds (Lazaridou et al., 2001; Rizzo, 2004; Goncalves & Romano, 2005 *in* Iipumbu 2008). It is used as stabilizer, gelling agent, fixer in different areas such as food (cheese, soups, sauces ...), cosmetics (creams, toothpaste ...), pharmaceuticals (medicines, syrups ...), paints, polishes, ceramics and adhesives (Batlle &Tous 1997).

- Other uses: The flower is used by beekeepers to produce honey locust honey or autumn. The bark and roots are used in the tanning industry thanks to their tannin content (El Kahkahi et al., 2016). The wood of the carob tree is used for production of furniture and as firewood.

Chapter 2:

Material and methods

1. Sampling site

Leaves of carob trees (*Ceratonia siliqua* L) were collected in June 2021 from Thala thestane (Cheurfa) village (266 m above the sea level, 36°47'53.48"N, 4°11'28.14"E) in Tigzirt district (Fig. 9), the village located at 4 km of Tigzirt town and 18 km of Tizi-ouzou province. The climate of this site is humid (Stoutah, 2016), the presence of the sea and the vegetation contributes to the softening of the average temperatures which are of the order of 21.11° C. the rains extend from October to April, with a maximum of precipitation in December; annual precipitation can reach 952 mm and annual humidity is 69.66 % (Messaoudi & Dahmani, 2013).

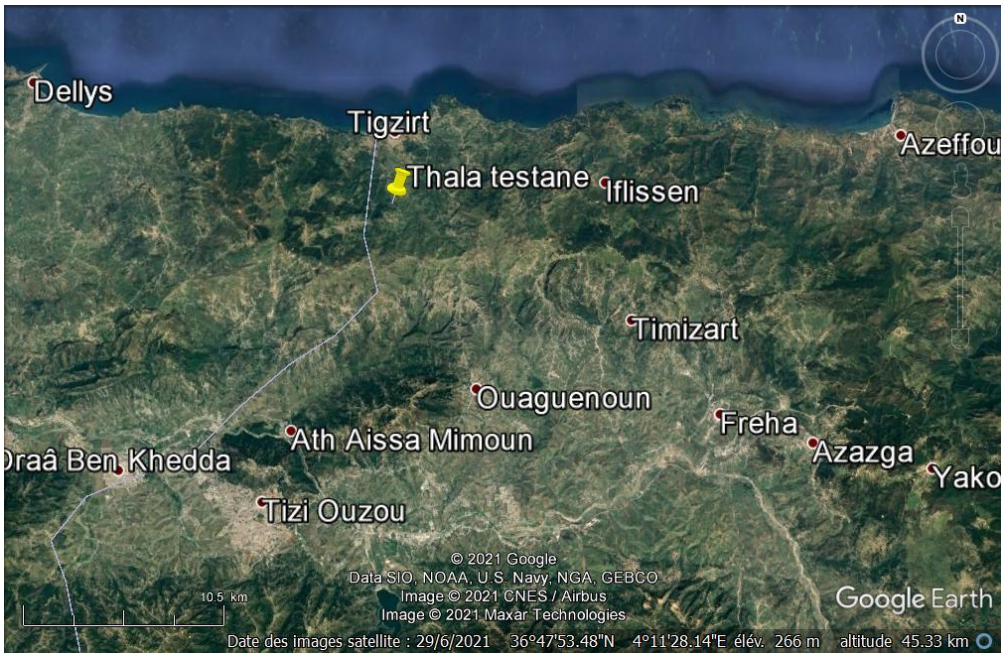


Figure 9: Location of sampling site (yellow pin) on a Google earth in June 2021.

2. Sampling and handling of biological material

Samples of 260 matures leaves (dark green) were randomly harvested from 26 adult trees (13 males and 13 females, 10 leaves per tree). The leaves were dried at ambient temperature of the laboratory and kept as vouchers in paper bags until measurements.

3. Leaf morphological traits

3.1 Quantitative traits

We followed the method described in the International Plant Genetic Resources Institute (Batlle & tous, 1997) for leaf characters. We measured: Leaf length (Ll), Leaf width (Lw), Petiole length (Pl), Rachis length (Rl), Leaflets number (Lfn), Basal leaflet length (Blfl), Basal leaflet

width (Blfl), Terminal leaflet length (Tlfl), Terminal leaflet width (Tlfw). The measurements were made using a ruler (Fig. 10).

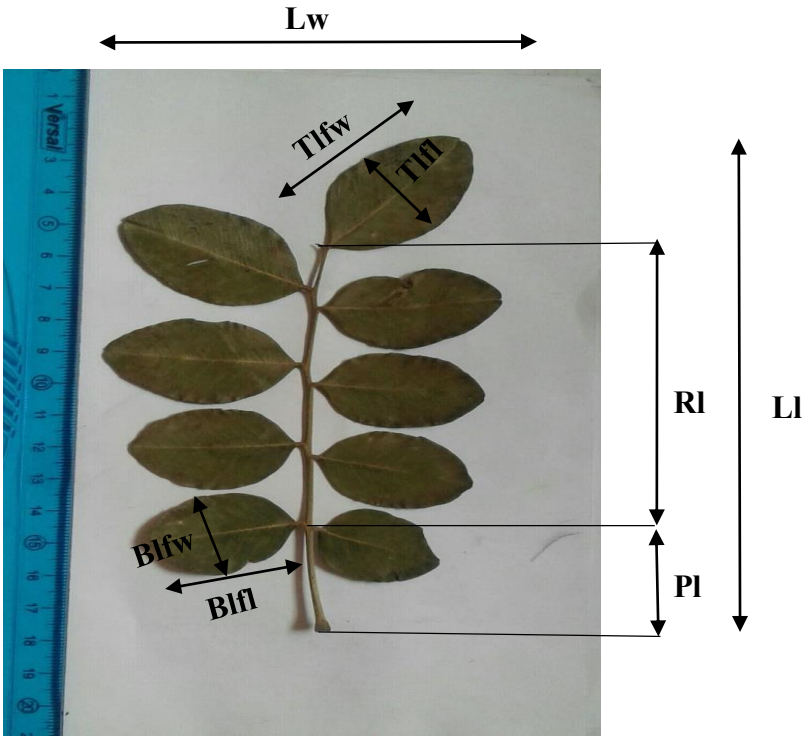


Figure 10: Measured traits on carob leaves.

3.2 Qualitative traits

As qualitative trait we recorded the presence or absence of the terminal leaflet.

4. Specific leaflet weight

Specific leaflet weight (Slfwe) measured by Mettler Toledo analytical balance with an accuracy of 0.0001g.

5. Number of stomata on the abaxial face

We counted the number of stomata in a 1 sq. mm under a light microscope (400× magnification).

6. Statistical analysis

Results were presented as mean ± SD (Min-Max) and bars chart. We used ANOVA to evaluate the effect of gender on leaf traits.

Chapter 3:

Results

III. Results

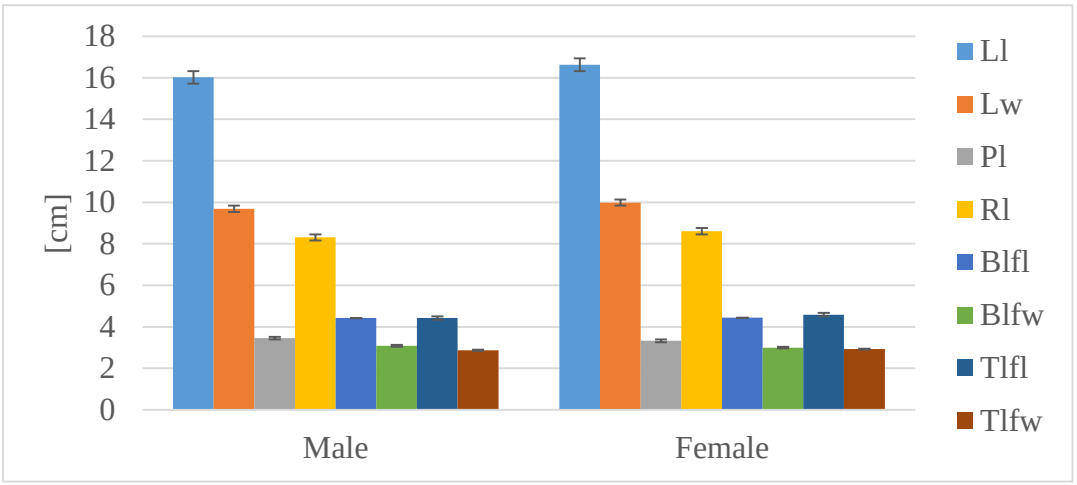
1. Male versus female number of stomata and leaf morphological traits

1.1. Descriptive statistics

1.1.1 Quantitative parameters

There was no difference in leaf size, petiole length, rachis length, the number of leaflets, basal leaflet size and terminal leaflet size (Fig. 11) between male and female trees. There was a marginal difference in specific leaflet weight (Fig. 12). When comparing number of stomata the female trees had higher number of stomata than male trees (Fig. 13).

A



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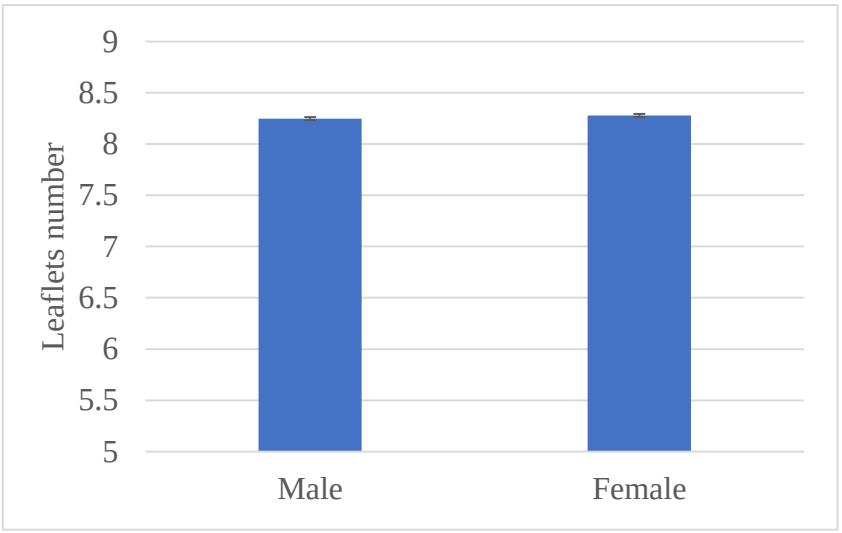

Figure 11: (A-B) Leaf morphological traits of male and female *Ceratonia siliqua* trees. Bars represent means ± 1 SE.

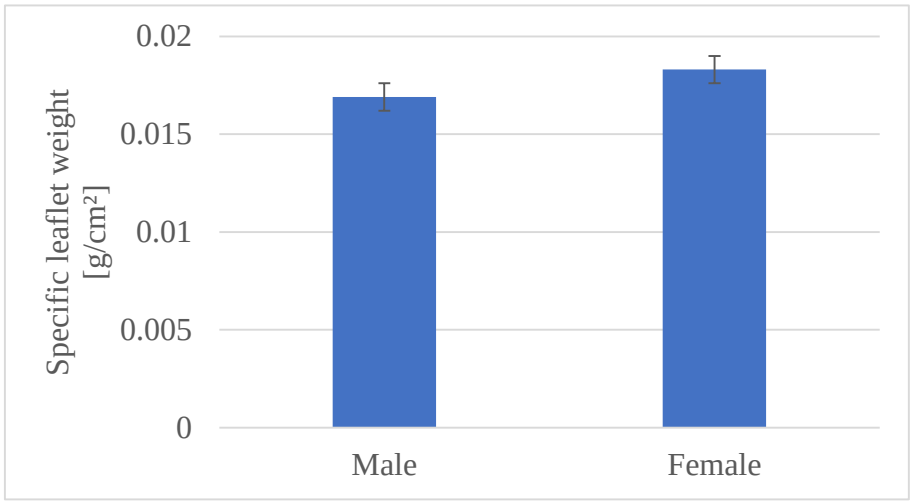

Figure 12: Specific leaflet weight in female and male *Ceratonia siliqua* trees. Bars represent means ± 1 SE.

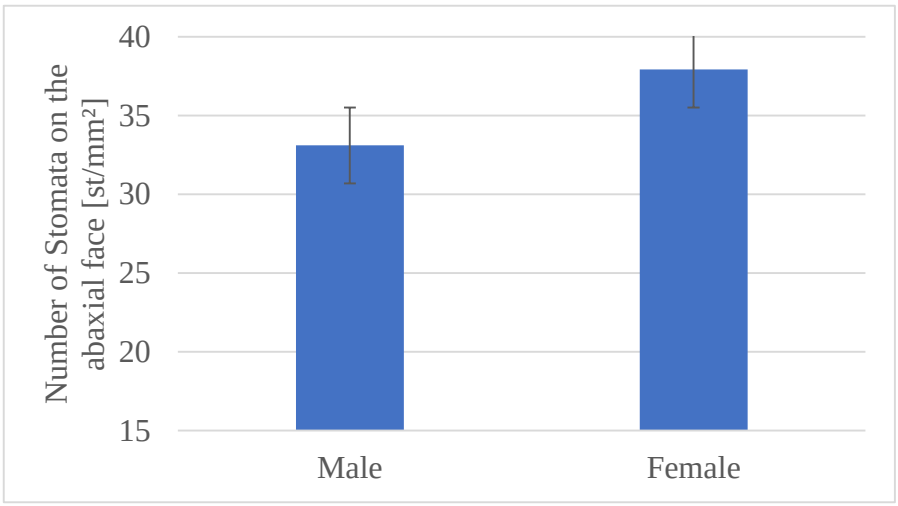

Figure 13: Number of stomata of male and female *Ceratonia siliqua* trees. Bars represent means ± 1 SE.

1.1.2. Qualitative parameters

1.1.2.1. The presence or absence of the terminal leaflet

A. Presence of a terminal leaflet in males

90.76% of leaves are paripinnate, while only 9.23% of leaves had terminal leaflet (Fig. 14).

B. Presence of a terminal leaflet in females

86.15% of leaves were of the normal paripinnate type, while only 13.84% of leaves had terminal leaflet (Fig. 14).

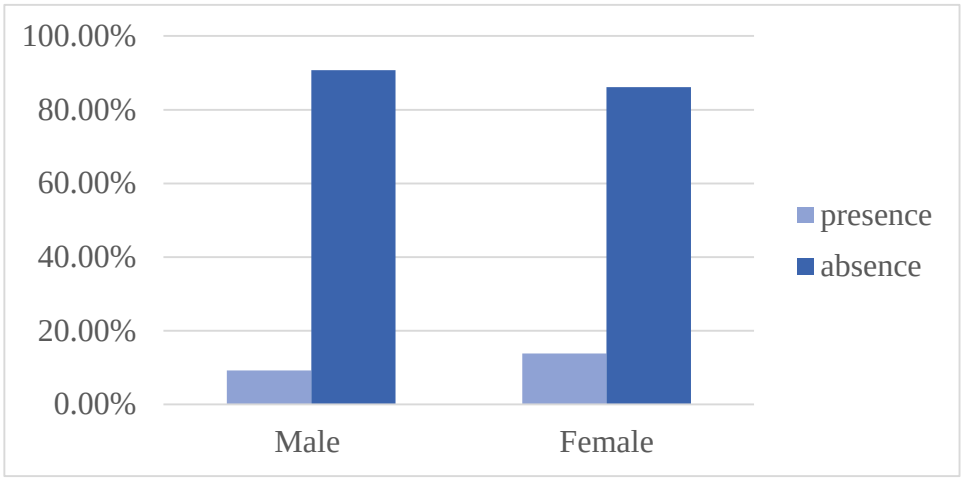

Figure 14: percentage of the presence or absence of terminal leaflet in female and male trees of *Ceratonia siliqua*.

2. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) shows a very highly significant difference in the number of stomata between male and female trees, while no significant differences were found in Ll, Lw, Pl, RL, Blfl, Blfw, Tlfl, Tlfw, Slfwe. (Table 2).

Table 2: Statistical parameters of leaf morphological traits and number of stomata for male and female trees of *Ceratonia siliqua*.

	Mean±SD/Min-Max			
Parameters	Overall	Male	Female	Gender
Leaf length (cm)	16.33 ± 3.28	16.02 ± 3.38 9.9 - 24.5	16.64 ± 3.16 10 - 27.7	ns
Leaf width (cm)	9.84 ± 1.93	9.69 ± 1.70 5.6 - 14	9.98 ± 2.13 5.4 - 19.5	ns
Petiole length(cm)	3.39 ± 0.95	3.46 ± 1.05 1.4 - 7.1	3.33 ± 0.84 1.5 - 6.2	ns
Rachis length (cm)	8.46 ± 2.21	8.31 ± 2.27 3.1 - 14.5	8.61 ± 2.15 4.3 - 13.6	ns
leaflets number	8.26 ± 1.44	8.25 ± 1.42 5 - 12	8.28 ± 1.48 4 - 10	ns
Basal leaflet length (cm)	4.43 ± 0.95	4.43 ± 0.93 2.6 - 6.8	4.44 ± 0.98 2 - 7.2	ns
Basal leaflet width (cm)	3.04 ± 0.62	3.09 ± 0.67 1.8 - 4.7	3 ± 0.57 1.9 - 4.6	ns
Terminal leaflet length (cm)	4.42 ± 1.15	4.20 ± 0.78 3 - 5.7	4.59 ± 1.37 3 - 7.7	ns
Terminal leaflet width (cm)	2.86 ±0.77	2.79 ± 0.65 1.7 - 4	2.92 ± 0.87 1.5 - 4.5	ns
Specific leaflet weight (g/cm²)	0.02 ± 0.012	0.02 ± 0.00 0.01 - 0.03	0.02 ± 0.01 0.01 - 0.12	ns
Number of stomata on abaxial face (st/mm)	35.51 ± 6.46	33.10 ± 5.96 21 - 52	37.92 ± 6.11 25 - 49	<0.001

Chapter 4:

Discussion

IV. Discussion

We found the overall mean of leaf length and width, petiole length, rachis length, number of leaflets, basal leaflet length and width, terminal leaflet length and width were 16.32, 9.83, 3.39, 8.76, 8, 4.43, 3.04, 4.42, 2.86 cm, respectively. In the literature, there was a limited report about leaf dimensions of carob because most of the morphological studies are concentrated on pod and seed characteristics. Seghire et al. (2016) found mean leaf width and length, petiole length and the number of leaflets of carobs in Morocco as 14.5 cm, 10.9 cm, 4.12 cm, 7 leaflets respectively. They also reported average leaflet length and width as 5.56 cm, 3.59 cm respectively indicating similarities with our results.

Our results comparing the difference between male and female leaves (descriptive analysis) differ from previous study conducted by Gharnit et al (2005) on three categories of carob tree from the province of Chafchaouen (NW of Morocco) as they basing on a descriptive analysis found that male leaves are longer and wider and have longer petiole and the leaflets are also longer, wider and more numerous in males compared to females, contrary to our results which showed similar values between males and females. However, our study indicates that female trees had a higher number of stomata, but such aspect is poorly documented in the literature.

The same results obtained with ANOVA which showed significant differences in in the number of stomata between male and female trees (females had higher number of stomata than males). A similar situation is found in female *Taxus baccata* trees in which had a statistically significantly higher number of stomata rows and stomatal density (Iszkuło et al., 2009). These authors suggested that higher number of stomata may result in a less conservative water economy for female plants, which demand higher water resources than males. However in humid environment the higher water use by female is constrained by low evaporative (Hultine et al, 2007). Hultine et al, 2007 also found in humid environment female *Acer negundo* have higher stomatal densities on abaxial leaf surfaces and higher transpiration rates than males, which means that female trees are more productive. Therefore our female trees tend to have higher number of stomata (more stomata number provide more pores for transpiration) than males because of their high production effort (the higher energy cost for fruit and seed production). On the other hand XU et al (2008) found that in well watered conditions no significant differences in morphological traits (leaf area, total number of leaves, specific leaf area) between male and female of *Populus cathayana,* our results are obtained on males and female growing in a similar habitat and most of leaf traits are similar except the number of stomata which is slightly higher in females (the difference between them only 4 stomata).

Conclusion

Conclusion

Overall, this study demonstrates that genders of *Ceratonia siliqua* do not differ in leaf morphology (leaf length and width, petiole length, rachis length, number of leaflets, basal leaflet length and width, terminal leaflet length and width, specific leaflet weight), no sexually dimorphic traits were found except in the number of stomata (micromorphology) which is higher in females.

In complementarity with this present study, one may investigate gender effect on other leaf morphological traits (leaflet area, leaflet thickness and leaflet mass) as well as on physiological ones (photosynthetic activity, transpiration rate...) and we may also take into account some other descriptors on tree (height, stem size, growth…). In addition more sites may be needed to confirm the observed trend. Given the economic and ecological importance of the carob tree and the perspective of its plantation at large scales, it would be interesting to discriminate between male and female at an early stage in order to ensure adequate sex ratios in plantations. And molecular markers may be useful in this regard. Indeed such markers are expected to facilitate breeding programs by extracting the DNA from leaves and using RAPD marker to identify the sexes of carob tree.

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