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Pomological characterization of Carob tree (*Ceratonia siliqua* L.) from the Province of Sefrou, Middle Atlas of Morocco

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Abstract

Forty five Sefrou carob populations from three sites were studied to assess their genetic variation based on pod and seed measures. Locust beans were characterised by: length, width, thickness, chord, weight, number and weight of seeds, aborted seeds rate and seeds yield in addition to the twist coefficient. For seeds, we measured the length, width, the ratio "width/length", the thickness, the ratio "width/thickness", the weight and the yields of the various parts of the seed i.e., the endosperm, the embryo-cotyledons and the tegument. The results showed that the chord, the weight and the yields of seeds and pulp of the carob pod, and the seed weight allow a better discrimination between the three surveyed locations. However, there were no differences between the three localities regarding the width and thickness of the carob pod, and the seed tegument yield. Generally, the intra-locations changes show a very highly significant difference between the trees of each location for all the parameters studied. These results show that environmental conditions do not explain alone the variation in pomological parameters followed and thereby there would be probably a genotypic variability within each locality.

Keywords: Pomological characterization, Carob tree, Carob pod, Carob seed, *Ceratonia siliqua* L., Sefrou, Morocco.

Introduction

The carob tree (*Ceratonia siliqua* L., *Fabaceae*) has an increasingly socio-economic and ecological interest in the world seeing various socioeconomic uses of gum extracted from the carob seed, flour obtained from locust bean (food, pharmaceutical, film, textile, cosmetics…) and carob tree in reforestation against desertification and soil erosion (Correia & Martins-Loucao**,** 2005). Originally from the Middle East and the Southwestern Asia, its distribution currently covers the five continents, particularly under the Mediterranean climate (Northern Africa, Middle East, Southern Europe, Canary Islands and more recently in Australia, Southern Africa, the USA, India and Southern America) (Tous *et al*., 1996; Battle & Tous, 1997; Yousif & Alghzawi, 2000; Gharnit & Ennabili, 2016).

The annual world production of carob bean is about 315,000 tons produced from approximately 200,000 hectares with variable yields depending on the cultivar, region, and agricultural practices (Makris and Kefalas, 2004). The main producers of carob bean and seed are Spain (36%-28%), Morocco (24-38), Italy (10-8), Portugal

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(10-8), Greece (8-6), Turkey (6-4), Algeria (4-3), Cyprus (3-2) and Tunisia (1-1) in the same order (Ait Chitt *et al.*, 2007). The world demand for carob gum corresponds to approximately 35, 000 tons of seeds (Catarino, 1993; Roukas, 1994).

The characterization of carob cultivars or categories was led in Mediterranean productive areas based amongst other on carob pod and seed parameters (Coit, 1967; Orphanos & Papaconstantinou, 1969; Casanova *et al.*, 1987; Albanell *et al.*, 1988, 1996; Caja *et al.*, 1988; Crescimano *et al.*, 1988; Russo & Polignano, 1996; Gharnit, 2003; Barracosa & Cravador, 2006; Gharnit *et al.*, 2006b; Barracosa *et al.*, 2007; Naghmouchi *et al.*, 2009; Sidina *et al.*, 2009; Karababa & Coskuner, 2013; Mulet *et al.*, 2015).

In Morocco, the carob tree culture is experiencing a resurgence of interest in recent years because of its many applications in the food industry on the one hand and profitability distinguished compared to other fruit species and cereals in un-irrigated land (or Bour) on the side. The carob bean production would depend mainly on rainfall, reaching 16,000 tons of locust beans (4,800 tons of carob kernels) (ORMVAT, 2005).

Carob beans are sold in situ for 3 to 9 Moroccan Dirham (MAD)/kg, depending on the season and the region. The economical income overtakes 3,840 MAD/exploitant.year (e.g. Gharnit *et al.*, 2001, 2006a; PNTTA, 2007). In Morocco, there are some twenty crushing, processing and/or gum production units, with a global capacity surpassing 80,000 tons. Carob seeds are sold for 22 to 32 MAD/kg. Currently, there are three carob sale regions: Marrakech, Agadir and Fez (PNTTA, 2007).

Commercial transactions value of carob kernels far exceeds that of wood production. Carob pod, pulp, seed and gum are subjected to significant trade towards Europe. In 2003, the volume of exports in this regard was of 11,352 tons of flakes, meal and flour (i.e. 22.04 Millions MAD), 10,409 tons of locust beans (191.67 M MAD) and 2,037 tons of mucilage and thickeners (135.58 M MAD). Carob and carob by-products import for the same year reached 897 tons of locust beans (18.65 M MAD) and 54.9 tons of mucilage and thickeners (1.85 million DH). The trade balance in the sector is of 328.79 M MAD for export (MAPM, 2007).

Although studies have interested the carob tree for several provinces of Morocco (e.g. Ouchkif, 1988; Gharnit, 2003; Gharnit *et al.*, 2001, 2003, 2004, 2005, 2006, 2010; Sidina *et al.*, 2009; El Hajaji *et al.*, 2011; Hasib & El Batal, 2014; El Kahkahi *et al*, 2014), to our knowledge, carob from the province of Sefrou was never reported or investigated. This work aims to characterize carob tree from the Sefrou province (Middle Atlas of Morocco) using specifically pod and kernel parameters.

Materials and methods

Study area

The Sefrou Province (Middle Atlas of Morocco) is geographically located between the Saïs plain and the Northern foothills of the Middle Atlas. It covers an area of 4,009 km^2, i.e. 19.7 % of the area of the Fès-Boulemane Region (HCP/RGPH, 2004). The area of arable land is estimated at 95,000 ha, of which 15,000 ha are irrigated. The forest occupies nearly 140,000 ha. The substrate of this area is dark calcareous with diversity in its constitution (granite and/or red sandy-granite in the Atlas Mountains; CRFB/MHUPV, 2013).

This Region is characterized by a continental climate with cold winters and dry and hot summer in the Northern part, wet and cold in the mountainous areas of central and semi-desert in the highlands near the Southern Province of Boulemane. The average minimum temperature of the

coldest month is about 8.9°C, while the average maximum temperature of the warmest month is 33.7°C. The temperature can exceed 40°C especially during the summer period and when the "Chergui" winds dominate. Moreover, the average annual rainfall is 648 mm. The snow appears from altitudes of 1600 m (CRFB/MHUPV, 2013).

The Sefrou Province shelters a variety of species in its forest heritage, thanks to the diversity of ecological conditions that characterize its territory: holm oak, cedar, arborvitae and juniper. The distribution of these species is a function of altitude: *Salsolaceae* in the lower altitudes, alfa and rosemary from 1,000 to 2,000 m of altitude, and Aleppo pine, cedar, red cedar and juniper cade in the higher altitudes (CRFB/MHUPV, 2013).

The Sefrou Province houses a population of 259,577 inhabitants, with 56% of rural population and a density of 67 inhab./Km^2 (RGPH, 2004). Its economy is based on livestock and orchards. The main fruit trees cultivated and/or exploited are olive, apple, plum, cherry, walnut, almond and carob tree (DRA, 2010).

Within the study area, the spontaneous carob irregularly covers fruit orchards. According to a survey carried out in 2010, three sites in the Province of Sefrou, where the carob tree grows in large populations, have been identified: Sekoura M Daz, Zair Sid Lahcen and Kandar Sidi Khiar.

Sampling locations

In 2010, three stations were selected from others to carry out systematic sampling of the carob tree at the Province of Sefrou, Fès-Boulmane Region:

1. Centre Sekoura - M Daz (1013 m, 04° 32'48,9804" W, 33° 31'34, 6872" N), with orchards based on carob and olive terraces low slope (10%), a western exposure and a limestone and conglomerate substrate.

2. Oued Guigou Sekoura - M Daz (991 m, 04° 33'39,654" W, 33° 31'15, 4956" N), proximal to the Centre Sekoura - M Daz, with orchards abandoned made of carob and olive, and crossed by irrigation canals, steep slopes (60-70%) and variable exposure to soil sufficiently developed and sandstone substrate.

3. Kandar Sidi Khiar (989 m, 04° 59'37,593" W, 33° 47'16,455" N), corresponding to a degraded Ceratonaie (development of *Urginea maritima*, *Arisarum vulgare*, *Thymus* spp., *Salvia* spp., and *Asphodelus* spp.; grazing...), medium slope (40%) and a variable exposure, and a limestone substrate.

Methods

For each of the three selected location, and following field surveys, a series of carob trees has been identified (14-16 trees), and samplings of 30 locust beans and 30 carob kernels per tree were systematically carried out.

The carob pod was characterized by the following parameters: length, width, thickness, chord, weight, seeds number and weight, rate of abortion and yield of seeds (Gharnit *et al.*, 2006b), and the twist coefficient.

We measured also in carob seed the length, width, thickness, "width/length" and "width/thickness" ratios, weight and yields of its different parts namely the endosperm, embryo-cotyledons and tegument (Gharnit *et al.*, 2006b).

All data were processed using the statistics 5.0 software (Statsoft Inc., 1995).

Results and Discussion

Carob pod

The chord, weight and yields of seeds and pulp of the carob bean show a high discrimination between all surveyed locations (Table 1). It is the same for (i) the pulp weight: 4.22 ± 1.72, 3.84 ± 1.47 and 5.33 ± 2.16 g (F ratio = 77.321, P = 0); (ii) seeds weight: 1.65 ± 0.48, 1.69 ± 0.45

and 1.90 ± 0.54 g (F ratio = 30.268, P = 1.451 E-13); and (iii) the number of aborted seeds: 0.28 ± 0.56, 0.41 ± 0.72 and 0.23 ± 0.57 g (F ratio = 9.852, P = 5.680 E-05) respectively in Centre Sekoura, Oued Guigou-Sekoura and Kandar Sidi Khiar.

The length, twist coefficient, and seeds number of the carob bean classify the prospected locations in two groups: Centre Sekoura and Oued Guigou-Sekoura on the one hand and Kandar Sidi Khiar on the other hand. While the aborted seeds rate gathers Centre Sekoura and Kandar Sidi Khiar on one side and Oued Guigou-Sekoura on the other. However, the width and thickness of the carob bean did not allow any distinction between these locations (Table 1).

Intra-locations variation (95% ANOVA) demonstrates a very highly significant difference between the trees of each location for all the parameters studied in carob bean, except for the twist coefficient (difference not significant) and seed abortion (significant difference) in Oued Guigou-Sekoura. Environmental conditions would not explain alone the variation of measured parameters in carob bean.

In location, seeds yield is highly and positively correlated ($p < 0.05$) with the length, width, thickness, chord and twist coefficient of the carob bean. It is the same in the tree.

The pulp yield is very highly correlated with the seeds number in the location, and with the seeds number and aborted seeds in the tree. Always considering the tree, the tegument yield is highly correlated with both the ratio of width and length of the carob bean.

Compared with carob bean from the province of Chefchaouen, that from Sefrou Province is longer (14.0-15.8 vs. 12.2-14.5 cm), narrower (1.46-1.51 vs. 1.88-2.3 cm), thinner (0.54-0.57 vs. 0.63-0.78 cm), straighter (ration chord to length of carob bean: 0.70-0.77 vs. 0.67-0.76) and lightest (5.53-7.21 vs. 8.03-11.8 g), and with a lower aborted seeds rate (2.05-4.02 vs. 2.11-6.20%), a higher seeds yield (28.1-32.1 vs. 16.6-22.0%) and a less important pulp yield (vs. 67.9-72.3 77.6- 83.5%) (Table 1; Gharnit *et al.*, 2006b). By comparison to carob from Beni Mellal, another region of Midle Atlas of Morocco, carob pods from Sefrou Province are longer (14.68 vs.11.99 cm), narrower (1.50 vs. 1.54 cm), thinner (0.56 vs. 0.53), and has a higher seeds yield (29.99 vs. 25.06 %) and more seeds (11.26 vs. 10.81) (Table 2; Sidina *et al.*, 2009)

Carob seed

The seed weight is one of the most discriminatory parameters between the three locations (Table 2). It is the same for the endosperm weight (0.065 ± 0.018, 0.069 ± 0.014 and 0.074 ± 0.016 g; F ratio = 30.031, P = 1.818E-13) and the tegument one (0.019 ± 0.006, 0.020 ± 0.007 and 0.023 ± 0.009 g; F ratio = 31.29, P = 5.473E-14) respectively in Centre Sekoura, Oued Guigou-Sekoura and Kandar Sidi Khiar.

Based on the width, “width/length” ratio, thickness and “width/thickness” ratio of the seed, the localities surveyed congregate into two groups: Centre Sekoura and Oued Guigou-Sekoura on one hand and Kandar Sidi Khiar the other (Table 2). It is the same for the embryo-cotyledons weight (F ratio = 30.005, P = 1.863E-13).

The seed length, and endosperm and embryo-cotyledons yields enable a distinction between Centre Sekoura and Kandar Sidi Khiar on the one hand, and the Oued Guigou-Sekoura on the other hand. The tegument yield allows no differentiation between these locations.

The intra-locations variation (95% ANOVA) showed that the trees of the same locality were highly different for all seed parameters considered, confirming also the importance of the genotype in this regard when compared to the provenance (location).

Table 1. Carob pod characteristics according to locations. ANOVA to 95 %, N=1260. Values followed with the same letters are not significantly different.

Locations	Length (cm)	Width (cm)	Thickness (cm)	Chord (cm)	Twist coefficient	Weight (g)	Seeds Number	Seeds abortion (%)	Seeds Yield (%)	Pulp yield (%)
Center of Sekoura	14.165 ±2.514a	1.483 ±0.181ab	0.569 ±0.129a	10.511 ±3.179	0.398 ±0.988a	5.864 ±1.863	11.019 ±2.489a	2.605 ±5.382a	29.851 ±10.023	70.149 ±10.023
Guigou wadi, Sekoura	14.025 ±2.383a	1.455 ±0.213a	0.553 ±0.118ab	9.833 ±2.770	0.333 ±0.661a	5.529 ±1.511	10.818 ±2.386a	4.019 ±7.248	32.073 ±10.126	67.927 ±10.126
Kandar Sidi Khiar	15.845 ±2.809	1.506 ±0.248b	0.542 ±0.131b	12.179 ±3.952	0.75 ±1.225	7.214 ±2.325	11.96 ±2.726	2.053 ±5.193a	28.054 ±9.700	72.281 ±9.927
F ratio	65.018	5.887	4.853	55.229	21.763	89.863	24.22	11.934	17.186	19.801
P	1.350 E-27	0.003	0.008	1.238 E-23	5.110 E-10	0	4.774 E-11	7.342 E-06	4.328 E-08	3.413 E-09
Location effect	***	**	**	***	***	***	***	***	***	***

Table 2. Carob kernel characteristics according to locations. ANOVA to 95 %, N=1260. Values followed with the same letters are not significantly different.

Locations	Length (L - cm)	Width (W – cm)	"W/L" ratio	Thickness (T – cm)	"W/T" ratio	Weight (g)	Endosperm yield (%)	Embryo-cotyledons yield (%)	Seed coat yield (%)
Center of Sekoura	0.809 ±0.119a	0.628 ±0.092a	0.788 ±0.134a	0.396 ±0.061a	1.633 ±0.447a	0.108 ±0.020	59.297 ±9.762a	22.272 ±6.960a	18.431 ±6.215ab
Guigou wadi, Sekoura	0.781 ±0.098	0.624 ±0.089a	0.806 ±0.125a	0.397 ±0.042a	1.587 ±0.284a	0.113 ±0.017	60.785 ±7.434	21.176 ±5.839	18.039 ±5.785ac
Kandar Sidi Khiar	0.823 ±0.131a	0.698 ±0.117	0.874 ±0.223	0.387 ±0.072	1.899 ±0.651	0.125 ±0.021	59.082 ±8.744a	22.134 ±6.568a	18.784 ±6.764bc
F ratio	13.753	74.115	30.77	3.818	51.162	80.028	4.775	3.574	1.485
P	0.000001	0	9.00E-14	0.022	4.353E-22	0	0.009	0.028	0.227
Location effect	***	***	***	*	***	***	**	*	NS

Considering the location, the embryo-cotyledons and tegument yields of the seed are very highly correlated with the seed width. In the tree, the endosperm yield of seed is very highly correlated with the "width/thickness" ratio, and highly correlated with the "width/length" ratio of the seed. The embryo-cotyledons yield is very highly correlated with the length and thickness of the seed. The tegument yield is very highly correlated with the width, thickness and the "width/thickness" ratio of the seed.

By comparison to the carob seed from the Province of Chefchaouen, the carob seed from the Sefrou Province is shorter (0.781-0.823 vs. 0.926-0.951 cm), narrower (0.624-0.698 vs. 0.76-0.78 cm), thinner (0.387-0.397 vs. 0.429-0.461) and lightest (0.108-0.125 vs. 0.17-0.30 g), and with a higher endosperm yield (59.1-60.8 vs. 41.1 -50.1%), a higher embryo-cotyledons one (21.2-22.3 vs. 18.6-21.4%) and a less important yield of tegument (18.0-18.8 vs. 21.1-24.6%) (Table 2; Gharnit *et al.*, 2006b). When compared to the carob from Beni Mellal, the carob seeds from the Sefrou Province were slightly similar in length (0.80 vs. 0.83 cm), width (0.65 vs. 0.63 cm) and thickness (0.39 vs. 0.41 cm) but differed in weight (0.115 vs. 0.15 g) (Table 2; Sidina *et al.*, 2009).

Conclusion

As concluded in the first part of our study concerning the leaves and efflorescence (submitted for publication) of carob tree, the variation in carob pod and seed parameters seeing in this second part of our study could not be explained by the environmental conditions, although its importance thereby, it would largely have a genotypic origin. Thus, a genetic investigation may report an interesting complement to these studies.

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