Comparison of Morphological Characteristics of Carob Tree (*Ceratonia siliqua* L.) Pods and Seeds of Populations Collected from Two Distant Croatian Islands: Drvenik Mali and Mali Lošinj

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Summary

It is well-known that carob tree (*Ceratonia siliqua* L.) is not overspread in Croatian north Adriatic islands and coast. It is hardly possible to find spontaneous (not planted) trees of *Ceratonia siliqua* northern from the island of Dugi Otok and Zadar coast. During the first investigated year of research project TEUCLIC financed by Croatian Foundation for Science, we found the northernmost habitat of carob tree on the island of Mali Lošinj. This paper deals with the results of morphometric analysis of carob tree pods and seeds of Mali Lošinj population, compared with the same analysis of carob tree population of Drvenik Mali, island placed in middle-Dalmatian archipelago, in order to find the possible differences of most important morphological traits between these two relatively distant populations. After provided morphometric research the differences in length and thickness of carob pods between these two populations were not significant. However, the pods of carob trees of Mali Lošinj population had significantly smaller weight and smaller number of seeds per pod in the comparison with Drvenik Mali population. Also, width of carob pods of Mali Lošinj population was significantly smaller in the comparison with Drvanik Mali population, as well as weight and average number of seeds per pod. The carob seeds of Drvenik Mali population are significantly longer, wider and heavier in the comparison with the Mali Lošinj population, in spite of the fact that the seeds of population of Mali Lošinj were significantly thicker in the comparison with seeds of Drvenik Mali population. The differences between these morphometric traits cannot be explained by environmental conditions. Differences in morphometric traits of carob pods and seeds between these two populations are primarily caused by genetic factors, which have to be confirmed by the analysis of genomic DNA isolated from the leaves.

Key words

carob tree, *Ceratonia siliqua*, morphometric traits, environmental conditions, genetic factors

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Introduction

Carob tree (*Ceratonia siliqua* L.) 2*n*=24, is a plant belonging to the Fabaceae family (Ekinci et al., 2010). It is leguminous evergreen tree native to the coastal regions of Mediterranean basin and southwest Asia, and is considered to be an important component of vegetation for economic and environmental reasons (Karababa and Coşkuner, 2013). The ancient Greeks brought it from its native Middle East to Greece and Italy, and it was also disseminated by the Arabs along the North African coast and north into Spain and Portugal. Carob tree has been neglected with respect to both cultural practices and research and development. It was spread in recent times to other Mediterranean-like regions such as California, Arizona, Mexico, Chile and Argentina by Spanish, and also to parts of Australia by Mediterranean emigrants and to South Africa and India by the English (Akbulut and Bayramoglu, 2013; Rababah et al., 2013). In the 4th century A.D. the Roman and Byzantine weight for carat was based on the weight of the seed of the carob tree (Oddy and La Niece, 1986). However, the variability of pre-metrication carat weight standards is also around 5% suggesting that human rather than natural selection gave rise to the carob myth (Turnbull et al., 2006). Carob seeds are very important source of galactomannans (Carob gum - E410) that has an important role in food and pharmaceutical industry. Galactomannans are polysaccharides consisting of a mannose backbone with galactose side groups (more specifically, a (1-4)-linked beta-D-mannopyranose backbone with branchpoints from their 6-positions linked to alpha-D-galactose, i.e. 1-6-linked alpha-D-galactopyranose) (Prajapati et al., 2013).

Due to a breeding objectives for carob (Batlle and Tous, 1997) the aim of this research is to find the variability in morphological and micro morphological characteristics of carob pods and seeds between populations of two distant islands i.e. Drvenik Mali island and Mali Lošinj. Namely, the north border of habitats of spontaneous plants of carob tree on Adriatic islands and coast is surrounding of Zadar (Fig. 1). So, it is hardly possible to find even sporadic and spontaneous (not planted) trees of *C. siliqua* north from the island of Dugi Otok and Zadar coast. During the first research year of research project TEUCLIC (acronym for: Taxonomy, Ecology and Utilization of Carob tree and Bay

Figure 1. Locations of Drvenik Mali and Mali Lošinj and boundaries of carob tree (*Ceratonia siliqua* L.) growing area

Laurel in Croatia) financed by Croatian Foundation for Science, we found the northernmost habitat of carob tree on the island of Mali Lošinj. This paper deals with the results of morphometric analyses of carob tree pods and seeds of Mali Lošinj population, compared with the same analyses of carob tree population of Drvenik Mali, island placed in middle-Dalmatian archipelago, which is a typical natural habitat of carob tree population (Fig. 1).

Materials and methods

The samples of pods were collected from wild growing plants of carob tree on the islands of Drvenik Mali (basic coordinates: 44.45°N and 16.08°E) and Mali Lošinj (basic coordinates: 44.32°N and 14.28°E) during the stage of maturity in September of 2015. Altitude and latitude of every location of habitat were determined by GPS locator. The pods from randomly selected plants were collected in their harvest time. On location of island of Mali Lošinj 63 pods were collected from five carob trees, and on location of island of Drvenik Mali 130 pods were collected from 12 carob trees.

Standard morphometrical analyses were performed by using the analytical balance Mettler Toledo with two decimal places and calliper. The geometric mean diameter (Dg) of seeds was calculated using Equation (1) (Karababa et al., 2013; Mohsenin, 1980). Sphericity (ϕ) of carob seeds (Karababa et al., 2013; Mohsenin, 1980) was calculated using Equation (2):

$$Dg = (LWT)^{\frac{1}{3}} \quad (1)$$

$$\phi = \frac{(LWT)^{\frac{1}{3}}}{L} \quad (2)$$

where L is length, W is width and T is thickness in mm.

Also, carob seeds volume and surface area were calculated using Equations 3, 4 and 5 (Karababa et al., 2013; Jain and Bal, 1997).

$$V = \frac{\pi B^2 L^2}{6(2L - B)} \quad (3)$$

$$S = \frac{\pi B L^2}{2L - B} \quad (4)$$

Where B (Equation 5) is defined as the geometric mean of the second largest and the smallest principal dimensions of the grain (Jain and Bal, 1997).

$$B = (WT)^{\frac{1}{2}} \quad (5)$$

Simultaneously with sampling of plant material, the soil was also collected *in situ* from all locations. Soil sampling was provided with the soil probe, from depths of 0–15 cm, because of the very small depth of non-developed karst soil. The soil samples were prepared for chemical analyses according to ISO 11464:1994 method (Pernar et al., 2013). Statistical analysis was performed by standard descriptive statistics and one-way ANOVA for two independent samples, using the Statistica SixSigma software. Morphological analyses of plant sex of inflorescence were performed using a binocular magnifier Carl Zeiss – Stemi 2000-C at 2× magnification.

Results and discussion

All collected and analysed plants from both populations were bisexual (Fig. 2). Namely and unfortunately, in Croatia there is a bad custom of carob growers and collectors in free nature to destroy the male carob plants. In most of the cases carob growers grafts male on female carob plants in order to get bisexual plant of carob tree. Considering the results of analyses morphometric characteristics of carob pods it is obvious that the pods of carob trees of Mali Lošinj population has smaller weight and smaller number of seeds per pod in the comparison with Drvenik Mali population (D=15.4 g and 2.25; $P<0.00001$, respectively). Also, width of carob pods of Mali Lošinj population was significantly smaller in the comparison with Drvanik Mali population (D=8.66 mm; $P<0.00001$). On the other hand, there were no significant differences in length and thickness of carob pods between these two populations (D=2.67 cm and 2.28 mm; not significant) (Table 1).

Considering, the results of morphometric characteristics of carob seeds (Table 2), it is obvious that length, width and weight of carob seeds of Drvenik Mali population are highly significantly higher in the comparison with Mali Lošinj population (D=1.08 mm; D=0.69 mm and 0.05 g; $P<0.00001$, respectively), but thickness of carob seeds of population Mali Lošinj is significantly higher in the comparison with Drvenik Mali population (D=0.24; $P<0.00001$).

According to comparisons between standard morphometric characterization such as geometric mean diameter (Dg), their sphericity (ϕ), seeds volume (V) and surface area (S) the carob seeds of Mali Lošinj population are more spherical than the seeds of Drvenik Mali population (Table 3) (D=0.12). However, geometric mean diameter of seeds, volume of seeds and surface area of seeds of Drvenik Mali population are higher in the comparison with seeds of Mali Lošinj population (Table 3). Differences in morphometric traits of carob pods and seeds between these two populations are primarily caused by genetic factors, which have to be confirmed by the analysis of genomic DNA isolated

Figure 2. Inflorescence of bisexual carob tree (*Ceratonia siliqua* L.) plant

Table 1. Differences in morphometric characteristics of pods between two different populations of carob tree (*Ceratonia siliqua* L.) (df=191)

Comparison Drvenik Mali vs. Mali Lošinj	Drvenik Mali Mean±SD	Mali Lošinj Mean±SD	Difference	Probability t-test	Probability F-test
Length of pods (cm)	17.61±1.66	14.94±1.86	2.67 (ns)	-	0.272
Width of pods (mm)	26.48±1.34	17.82±2.13	8.66**	<0.00001	0.00001
Thickness of pods (mm)	11.62±1.18	9.34±1.20	2.28 (ns)	-	0.876
Weight of pods (g)	28.93± 4.38	13.53± 5.73	15.4**	<0.00001	0.011
Number of seeds per pod	11.05±1.86	8.80±3.12	2.25**	<0.00001	0.000003

(ns) – not significant, ** - significance higher than 99.99 %.

Table 2. Differences in morphometric characteristics of seeds between two different populations of carob tree (*Ceratonia siliqua* L.) (df=1731)

Comparisons Drvenik Mali vs. Mali Lošinj	Drvenik Mali Mean±SD	Mali Lošinj Mean±SD	Difference	Probability t-test	Probability F-test
Length of seeds (mm)	9.22±0.68	8.14±0.98	1.08**	<0.00001	<0.00001
Width of seeds (mm)	6.93±0.56	6.24±0.76	0.69**	<0.00001	<0.00001
Thickness of seeds (mm)	3.92±0.49	4.16±0.74	-0.24**	<0.00001	<0.00001
Weight of seeds (g)	0.19±0.14	0.14±0.05	0.05*	0.049	0.027

(ns) – not significant, ** - significance higher than 99.99%, * - significance higher than 95%.

Table 3. Standard morphometric characterization of carob seeds of Drvenik Mali and Mali Lošinj populations

Population	Geometric mean diameter of seeds – Dg (mm)	Sphericity ϕ	Volume of seeds – V (mm^3)	Surface area of seeds – S (mm^2)	B
Drvenik Mali	5.97	0.69	83.87	98.30	5.12
Mali Lošinj	5.85	0.71	80.24	94.62	5.09
Difference	0.12	-0.02	3.63	3.68	0.03

from the leaves. However, it is very important fact that growing of carob trees is possible even north of the boundary of distribution of carob tree, which depends on micro-climatological effects and exposition of some bays, which is probably case with the location of Mali Lošinj.

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