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GENETIC RESOURCES OF CAROB TREE (*CERATONIA SILIQUA* L.) IN ALGERIA: INSIGHT FROM POD AND SEED MORPHOLOGY

KOCHERANE Rima [1]*, KROUCHI Fazia [2] and DERRIDJ Arezki [2]

Department of Agronomy, Faculty of Nature and life Science, ZIANE Achour University, Djelfa, 17000, Algeria.
Laboratoire de production, amélioration et protection des végétaux et des denrées alimentaires, Department of Agronomic Sciences, Faculty of Biological and Agronomic Sciences, Mouloud MAMMERI University, Tizi-Ouzou, 15000, Algeria

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Abstract

Description of the subject. In Algeria, the carob tree extends over a large area from East to West. Yet data on the pattern of morphological variation of its pods and seeds across such area are lacking, despite the usefulness of a good understanding of intraspecific variation of such traits for domestication, conservation and sustainable management purposes.

Objective: The present study aims at describing morphological variation in pod and seed traits of such populations and addresses the following questions: (a) what is the level of variation within and between wild carob populations; (b) and how is such variation spatially distributed?

Methods: 07 wild carob populations of northern Algeria were investigated for pod and seed morphology.

Results: Significant differences between provenances were observed for all quantitative traits. Slight differences between trees were also observed. Pod shape and seed surface were the most uniform traits among provenances. Within-provenance, the proportion of polymorphic trees for a given qualitative trait was variable. The pattern of variation did not reflect ecological conditions at site of origin suggesting rather a mosaic of variations.

Conclusion: This study provides a first insight into carob genetic resources in Algeria through morphological characters of pods and seeds. It indicates a good potential of Algerian populations as a source of raw material for future breeding programs.

Keywords: *Ceratonia siliqua* L., Algeria; morphology; pod; seed; provenance; variation.

RESSOURCES GÉNÉTIQUES DU CAROUBIER (*CERATONIA SILIQUA* L.) EN ALGÉRIE: APERÇU Á TRAVERS LA MORPHOLOGIE DES GOUSSES ET DES GRAINES

Résumé

Description du sujet: En Algérie, le caroubier s'étend sur une vaste zone allant d'Est en Ouest, mais on manque de données sur le patron de la variation morphologique de ses gousses et de ses graines dans cette zone, malgré l'utilité d'une bonne compréhension de la variation intra-spécifique de ces caractères pour la domestication; la conservation et la gestion durable.

Objectifs: La présente étude vise à décrire la variabilité morphologique des caractères des gousses et des graines de ces populations et aborde les questions suivantes: a) quel est le niveau de variation à l'intérieur et entre les populations du caroubier sauvage; (b) et comment cette variation est-elle répartie spatialement?

Méthodes: 07 populations spontanées du caroubier du nord de l'Algérie ont été étudiées pour la morphologie des gousses et des graines.

Résultats: Des différences significatives entre les provenances ont été observées pour tous les caractères quantitatifs. De légères différences entre arbres ont aussi été observées. La forme et l'aspect des surfaces des gousses sont les plus uniformes entre les provenances. Au sein des provenances, la proportion d'arbres polymorphes pour un caractère qualitatif donné est variable. Le patron de variation ne reflète pas les conditions écologiques du site d'origine et suggère plutôt une mosaïque de variation.

Conclusion. Cette étude fournit un premier aperçu des ressources génétiques du caroubier en Algérie à travers les caractères morphologiques des gousses et des graines. Elle indique un bon potentiel des populations algériennes comme source de matière de base pour les futurs programmes de sélection.

Mots clés: *Ceratonia siliqua* L., Algérie; morphologie; gousse; graine; provenance; variabilité.

* Auteur correspondant: KOCHERANE Rima. e-mail: rimrim53@ymail.com

INTRODUCTION

The Carob tree (*Ceratonia siliqua* L.) is one of the most characteristic species of the Mediterranean region[39]. It is a legume of the Fabaceae family and Caesalpiniaceae sub-family. It is a spontaneous or a cultivated tree that may reach a height of 5 to 15 m [50, 9]. Together with *Pistacia lentiscus* L. and *Olea europaea* L. var. *sylvestris*, this species forms one of the most characteristic associations of the lowest zone of the Mediterranean vegetation and is thus considered to be a climax community (*Oleo-Ceratonion*) [9]. The Carob tree is cultivated in the Mediterranean countries including Spain, Portugal, Italy, Morocco, Tunisia and Algeria [50, 1, 4, 9, 8, 54, 45, 59], in Turkey[11], in south Africa and USA [27].

In Algeria, carob tree has been reported to be very common in the Tell region by [39] and [50] and frequently cultivated in the Saharan Atlas [50]. The Carob tree is tolerant to salt stress and drought [15, 21, 20, 14, 22]. In Algeria, it grows on all types of soils except very moist ones and it resprouts abundantly [39]. In Morocco, it grows under arid, semi-arid, sub humid and humid bioclimates in their warm and mild variants and it is also adapted to all soil types [1]. Carob cultivation in marginal and prevailing calcareous soils of the Mediterranean region is important environmentally and economically [9]. The species may contribute to developing disadvantaged marginal areas of Morocco [27] and salted zones of northwestern Algeria particularly Relizane region [40].

Carob tree produces an edible sweet pod with a much appreciated pulp in the Mediterranean region. Besides its consumption as fresh, dry or transformed material, the pod represents a valuable source of forage for livestock and is largely used in food industry [10]. The fruit provides flour used in food industry for its polyphenols antioxidant properties and in industrial production of bioethanol[44]. Carob pod extracts are also used for ethanol production by *Saccharomyces cerevisiae* [63, 67, 25], citric acid production[44], β-mannanase production[66, 26] and for enhancement of lactic acid production by *Lactobacillus casei* [62].

Carob seeds contain a gum called locust bean gum (LBG) or "E410" used in food industry as a stabilizing and gelatinous agent in various products [6], as an additive into sugar-free, starch-free flour for diabetics[65], as drug ingredient in pharmaceutical industry [57, 49] and as a source of dietary fibers in foods [16].

Pods extracts play a role in suppression of intestinal parasites [43] in the treatment of diarrhea [56], in the treatment or control of hyperlipidaemia (high cholesterol in plasma) and have antiproliferative [53], antibacterian [42], antioxidant and anti-inflammatory properties [44].

Such examples of multipurpose uses illustrate the growing importance of carob products mainly pulp and locust gum likely to promote its cultivation, use and conservation.

Indeed, despite its economic importance, *Ceratonia siliqua* an agroforestry tree in North Africa[30] is underutilized in such region particularly in Algerian which occupies only the 8th rank for carob production [9, 24]. Lack of research on diversity assessment is one of the constraints in national programs to promote conservation and use of underutilized crop species [47] and characterization, evaluation and descriptors lists are among the required activities to overcome such constraints [47]. A better understanding of genetic diversity and its distribution is essential for its conservation and use[51]. Seed source choice is important in a restoration and management perspective [12].

Morphological characterization of carob pods and seeds around the Mediterranean region has been carried out on spontaneous populations of the species [35, 11]; on cultivars [4, 54, 8, 59, 38, 29] or both types [28, 18, 45, 23, 19].

Carob pod and seed traits (i.e. size and quality of pods, seed yield) have been reported by [9] to vary according to cultivars as a result of centuries of cultivation. Significant variations of pod and seed traits have also been reported on wild populations of the species [28, 35, 18]. Such variation is suggested to be under influence of environmental factors [28]; edapho-climatic site factors[18]; latitude [29] and/or agronomic practices in the case of varieties[18].

In Algeria, the carob tree, a component of the *Oleo-Ceratonion,* extends over a large area from East to West encompassing low and medium elevations and semi-arid and humid bioclimates.

Yet data on the pattern of morphological variation of its pods and seeds across such area are lacking despite the usefulness of a good understanding of the within- species variation of such traits for domestication, conservation and sustainable management purposes.
The present study aims at describing morphological variation in pod and seed traits of such populations and addressed the following questions: *(i)* what is the level of variation within and between wild carob populations; *(ii)* and how is such variation geographically distributed?

MATERIAL AND METHODS

1. Study sites

The study has been undertaken on 7 spontaneous carob populations in north Algeria which are Annaba, Jijel, Setif, Blida, Tipaza, Relizane and Tlemcen respectively from East to West (Fig. 1). Such populations are located at various elevations (including low land, hilly and mountainous landscape) and evolve under different bioclimates based on calculations made on data provided by National Meteorology Office of Algeria (Table1).

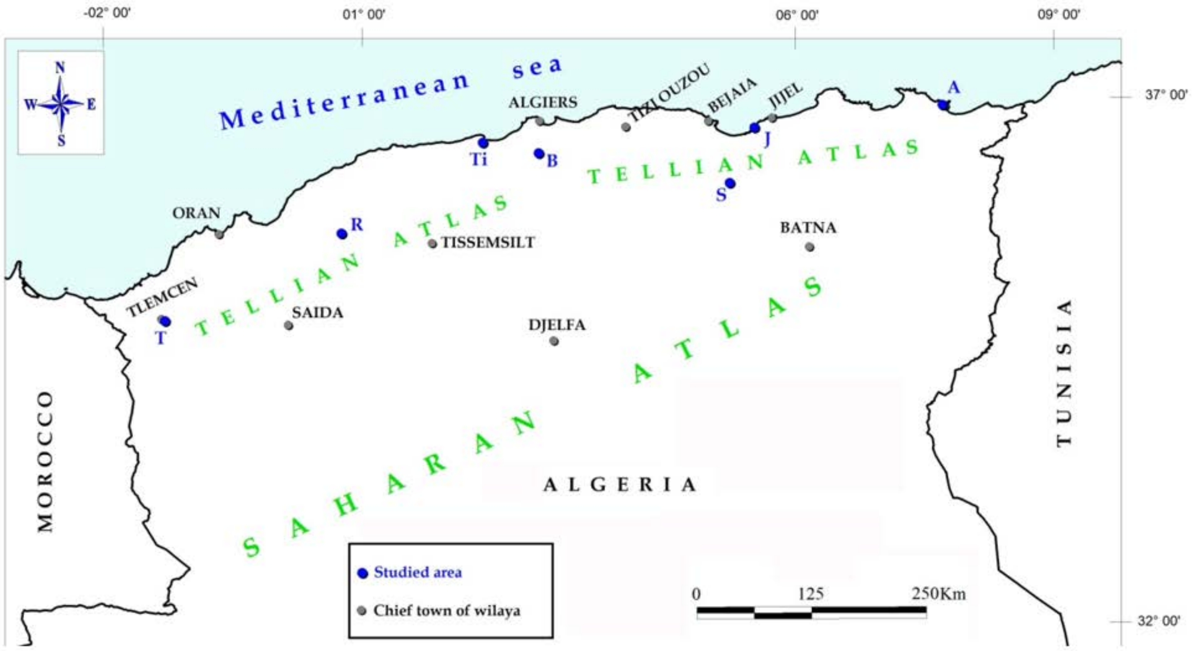

Figure 1. Map of Algeria showing locations of the carob sites analyzed.
*T:Tlemcen; R: Relizane; Ti: Tipaza; B: Blida; S:Setif; J:Jijel; A:Annaba.

Table 1. Geographic and climatic data of studied sites.

Site	Code	Latitude	Longitude	Altitude (m)	m (°C)	M (°C)	Rainfall (mm/year)	Q_3	Biolimate
Annaba	A	36°55'N	007°43'E	740	6.7	31.5	978.93	135.39	mild humid
Jijel	J	36°42'N	005°32'E	81	6.80	31.6	1044.1	144.41	mild humid
Setif	S	36°11'N	005°15'E	1050	1.92	34.30	414.5	43.91	fresh semi-arid
Blida	B	36°29'N	003°02'E	187	7	31.57	796.0	111.12	warm subhumid
Tipaza	Ti	36°34'N	002°24'E	32	9.50	32.1	574.8	87.24	warm subhumid
Relizane	R	35°42'N	000°46'E	155	5.8	32.7	402	51.26	mild semi-arid
Tlemcen	T	34°52'N	001°16'W	757	3.8	32.5	484	57.84	mild subhumid

Q3: Emberger quotient. M: Mean of the maxima temperature of the warmest month. m: Mean of the minima temperature of the coldest month.

2. Plant material

Pods and seeds used in this study were collected from the 7 carob populations during September and October 2014 coinciding with the peak maturity of pods. In each population, a random sample of 20 trees was retained and 20 pods per tree were randomly harvested, totalizing 140 trees and 2800 pods. The collected material was labelled with respect of tree and population identity then brought to the lab for measurements. The 20 pods of each tree were crashed of individually for seed extraction. Given the homogeneity of seed size within pods, one seed per pod was randomly retained for subsequent measurements totalizing 20 measured seed per tree and 2800 seeds in total.

3. Measured traits

A. Qualitative traits

Pods and seeds were characterized by their color, shape and surface aspect. Modalities of such qualitative traits were inspired from data literature[9].

B. Quantitative traits

Characterization of pods and seeds was made basing on descriptors of the International Plant Genetic Resources Institute “IPGRI” [9]. Pod and seed description were carried out on the basis of 14 variables namely: length; width; thickness chord; weight; pulp weight; total number of seeds per pod in the case of pods; length; width; thickness and total seed weight, mean seed weight in the case of seeds. Some synthetic variables were also calculated: ratio “pod length/pod width”; ratio “pod chord/pod length” and ratio “total seed weight/ pod weight × 100” corresponding to seed yield. Mean seed weight was calculated by dividing the total seed weight per pod on total number of seeds per pod.

In all 17 morphological characters were measured, of which 3 are qualitative and 14 are quantitative. Quantitative measurements were done using a caliper with a precision of ±0.1 mm and an electronic balance (SCALTEC SBA33, Heiligenstadt, Germany) with accuracy of ±0.1 mg. Qualitative measurements were based on naked eye observations.

4. Statistical analysis

In order to compare morphological variability of carob populations, data obtained on quantitative traits were subjected to statistical analysis.

A descriptive analysis (mean and coefficient of variation) was performed on quantitative traits followed by a two-way nested ANOVA. Linear correlations between variables were also calculated. Data obtained on qualitative traits were subjected to analysis of relative frequencies of modalities for each variable at three levels: on the whole sample, at provenance level and within-provenance. The Statistica10 software (Shareware) and Rx64.3.4.0 software were used for analyses.

RESULTS

3.1. Qualitative traits

Pod and seed qualitative traits (color, shape and surface aspect) and corresponding modalities are given in table 2.

Pod color : Three pod shade colors were identified among the whole sample of pods (Table2):dark-brown (45.97%), reddish-brown (28.36%) and light-brown (25.67%).

Light-brown pods were represented at a very high proportion in Relizane (i.e. 98.75%) and at an intermediate one in Annaba (40.75%). Dark-brown pods were dominant in Tlemcen, Jijel and Blida (i.e. 59.25; 80.50 and 90.50% respectively). Reddish-brown pods were better represented in Setif; Tipaza and Annaba (i.e. 57.75; 53.50 and 38.50% respectively) but were absent in Relizane and Blida and on all but one individual of Jijel.

Monomorphic individuals with exclusively light-brown pods were dominant in Relizane (95%). Monomorphic ones with exclusively dark-brown pods were dominant in Blida (85%); Jijel (70%) and Tlemcen (50%). Polymorphic individuals for pod color were dominant in Tipaza (100%); Annaba (65%) and Setif (60%).

Pod shape: Three pod shapes were recorded (Table 2): curved (90.07%); straight (6.29%) and spiral (3.64%).

At provenance level, curved pods were represented at high proportions across all provenances (Table 2) while spiral shape reached a maximum of 14.5% in Tlemcen and 10.25% in Annaba.

Within-provenance, monomorphic individuals for the straight or spiral pod shape were absent. Monomorphic ones for curved pod shapes were dominant in Relizane (80%) but were also present in Setif (55%) and Tipaza (45%).
Polymorphic individuals were dominant in Tlemcen; Jijel; Annaba; Blida where they accounted for 80 to 85%.

Pod surface: Three pod surfaces were recorded (Table 2): wrinkled (59.25%); smooth (40.11%) and very rough (0.64%).
At provenance level, wrinkled pod surfaces were better represented in Tlemcen, Jijel, Annaba, Relizane and Blida (91.25 to 56.75%) and smooth ones better represented in Tipaza i.e. 75.75%.
Within-provenance, a very rough pod surface was present only on 3 individuals in Tlemcen.
Individuals with an exclusively wrinkled pod surface were better represented in Tlemcen; Jijel, Annaba and Relizane (80; 65; 55 and 50% respectively) contrarily to Tipaza where they accounted for only 10%. Those with an exclusively smooth pod surface were absent in Tlemcen and best represented in Tipaza (60%) followed by Setif (50%). Polymorphic individuals for pod surface were best represented in Blida (60%).

Table 2: Distribution of trees for pod and seed qualitative traits.

Trait	Modalities	% of trees within-provenance (n=20)							% of total trees (n=140)
		Annaba	Jijel	Setif	Blida	Tipaza	Relizane	Tlemcen	
Pod color	Light -brown	40.75	14.5	0.25	0	19	98.75	6.50	25.67
	Dark-brown	20.75	80.5	42	90.50	27.5	1.25	59.25	45.97
	Reddish- brown	38.50	5	57.75	9.5	53.5	0	34.25	28.36
Pod shape	Straight	1.75	8	0	11	1.50	0.50	2.75	3.64
	Curved	88	85.75	97	85.75	92.75	98.50	82.75	90.07
	Spiral	10.25	6.25	3	3.25	5.75	1	14.50	6.29
Pod surface	Smooth	37.75	27.5	58	43.25	75.75	34.25	4.25	40.11
	Wrinkled	62.25	72.5	42	56.75	24.25	65.75	91.25	59.25
	Very rough	0	0	0	0	0	0	4.50	0.64
Seed color	Reddish brown	0	36.75	7.25	3.5	16.75	0	0	9.18
	Dark-brown	51.75	48	22.75	28.5	35.50	31	39.50	36.71
	Blakish-brown	28.25	5.25	0.25	0	0.50	3.50	1	5.54
	Light- brown	20	10	69.75	68	47.25	65.50	59.50	48.57
Seed shape	Rounded	59.25	31	96.25	10.25	22.75	96	49.75	52.18
	Oval	23.25	35.5	0.25	58.5	17.25	0	21.50	22.32
	Elliptical	17.50	33.5	3.5	31.25	60	4	28.75	25.50
Seed surface	Smooth	81.50	69.5	88.75	53.75	90	82.25	77	77.54
	Wrinkled	18.50	27.75	11.25	42	10	17.75	22	21.32
	Very rough	0	2.75	0	4.25	0	0	1	1.14

Seed color: 4 seed colors were identified (Table 2): light-brown (48.57%); dark-brown (36.71%); reddish-brown (9.18%) and blackish-brown (5.54%).
Light-brown seeds were dominant among 5 out of 7 provenances (Setif, Blida, Tipaza, Relizane and Tlemcen) with proportions ranging from a low of 47.25% to a high of 69.75% (Table 2). Dark-brown seeds were better represented in Jijel (48%) and Annaba (51.75%). Reddish-brown ones were absent among three provenances (Annaba, Relizane and Tlemcen).
Blackish-brown seeds were absent in Blida and did not exceed 5.25% in other provenances except Annaba where they reached a maximum of 28.25%.
The proportion of polymorphic individuals for seed color is higher than that of monomorphic ones for all provenances except Relizane and Blida which showed relatively balanced proportions of monomorphic and polymorphic trees.
Seed shape: Three modalities of this trait were observed (Table 2): rounded; oval and elliptical, which accounted respectively for 52.18; 22.32 and 25.50% of the whole sampled seeds.

Rounded seeds were highly represented in Relizane and Setif (96%) followed by Annaba (59.25%) and Tlemcen (49.5%); elliptical seeds were better represented in Tipaza (60%) and oval ones better represented in Blida (58.5%). Jijel contained these three modalities at comparable rates.

Polymorphic individuals for seed shape were best represented in Jijel; Tlemcen and Blida (65 to 60 %). Individuals with only rounded seeds were dominant in Setif (80%) and Relizane (70%), while those with only elliptical seeds best represented in Tipaza (40%).

Seed surface: Seed surface comprised three modalities (Table 2): smooth (77.54%), wrinkled (21.32%) and very rough (1.14%). The latter category is absent in 3 provenances (Tipaza, Setif and Relizane) and present at very low rates i.e. 1; 2.75 and 4.25% in Tlemcen; Jijel and Blida respectively.

For this trait, the proportion of polymorphic trees is higher than that of monomorphic ones for all provenances except Tipaza which contained 60% of monomorphic trees with an exclusively smooth seed surface.

3.2. Quantitative traits

Statistical parameters for pod and seed traits (i.e. overall means and corresponding coefficients of variation and range per tree) are given in Table 3. Boxplots of main traits are given in Fig. 2. The distribution of trees within-provenance for along with their mean values of pod and seed traits are given in Table 4 /Fig 3. The results of the two-way nested Anova on pod and seed traits are given in Table 5. The correlation matrix between traits is given in table 6.

3.2.1. Within and between provenance variations for quantitative traits

Pod length: The overall mean for this trait is 15.27 cm. The longest pods were observed in Tlemcen, followed by Annaba, and the shortest ones in Relizane followed by Jijel. All provenances contained individuals pertaining to the class of low and intermediate pod length even at fluctuating rates, while the class of longest pods was absent among individuals of Relizane, Jijel and Setif.

Pod width: The overall mean for this trait is of 1.97 cm. The largest pods were recorded in Setif; the narrowest ones in Jijel followed by Tipaza. 100% of individuals from Setif pertained to the class of the largest pods i.e.]2-2.5]cm and 100% of those from Jijel to the class of intermediate pod width i.e.]1.5 - 2] cm (Table 4). The remaining provenances contained individuals pertaining both to the class of intermediate and high values of pod width.

Pod thickness: The overall mean for this trait is of 0.76 cm. The thickest pods were found in Blida; the thinnest ones in Relizane. Overall, the intermediate class of pod width was recorded on 79% of individuals and was represented within each provenance. Contrary to the class of low values, it was recorded only among 25 and 35% individuals from Tlemcen and Relizane respectively and the class of high values recorded only among 35 to 45% of individuals from Annaba and Blida respectively.

Pod weight: The overall mean for this trait is 11.08 g. The heaviest pods were found in Blida, followed by Tlemcen, and the lightest ones in Relizane. As a whole, half of individuals pertained to the same class of mean values of pod weight. Individuals with low and intermediate pod weights were observed in each provenance while those with heavy pods were observed only in Blida, Tipaza and to a lesser extent in Tlemcen.

Total number of seeds per pod: In average there were 10.56 seeds per pod. The highest number of seeds per pod was recorded in Blida and the lowest one in Relizane. Indeed, Relizane contained a great proportion of individuals with no more than 8 seeds per pod, contrary to Blida and Tlemcen which contained a consistent proportion of individuals with more than 13 seeds/pod (Table 4/Fig. 3).

Seed yield: Overall, total seed mass represented 18.19% of pod weight. The highest mean was recorded in Tipaza and the lowest one in Relizane. Indeed, this parameter was below 10% among 80% of individuals from Relizane and exceeded 20% for 70% of individuals from Jijel and Tlemcen and for 55% of those from Blida and Tipaza.

Table 3: Statistical parameters on quantitative traits of pods and seeds.

Character	Pod											Seed			
	Length (cm)	Width (cm)	Thickness (cm)	Chord (cm)	Weight (g)	Pulp weight (g)	Seed number/pod	Total seed weight (g)	Seed yield (%)	Length / width	Chord/ Length	Mean seed weight(g)	Length (mm)	Width (mm)	Thickness (mm)
	pl	pwd	pth	pch	pwg	ppwg	tsn	swg	sy	pl/pwd	chr/pl	mswg	sl	swd	sth
Annaba	16.91	2.04	0.85	12.27	12.24	10.43	10.01	1.81	15.95	8.38	0.73	0,18	9.91	6.85	3.90
Jijel	13.64	1.76	0.81	11.50	9.38	7.45	11	1.92	21.88	7.78	0.84	0,18	8.86	6.65	4.28
Setif	15.36	2.34	0.66	11.52	11.48	9.99	9.32	1.49	13.32	6.57	0.75	0,16	8.90	6.98	4.14
Blida	15.46	1.98	0.90	12.50	14.30	11.57	12.76	2.73	21.34	7.81	0.81	0,23	9.76	6.82	4.34
Tipaza	14.66	1.84	0.78	11.72	10.25	8.20	11.01	2.04	23.98	8.02	0.80	0,19	9.33	7.09	4.19
Relizane	13.76	1.95	0.60	11.56	8.67	7.97	7.15	0.70	8.58	7.25	0.84	0,10	8.07	6.56	2.96
Tlemcen	17.08	1.91	0.68	12.63	11.18	8.84	12.63	2.34	22.55	8.94	0.74	0,19	8.98	6.86	4.44
Overall mean	15.27	1.97	0.76	11.97	11.08	9.22	10.56	1.86	18.19	7.83	0.79	0,17	9.12	6.84	4.04
CV (%)	18.47	14.91	22.18	21.28	36.20	40.67	29.54	41.89	49.51	20.42	17.03	33,13	10.05	7.66	15.18
Range per tree	10,09 - 20,59	1,02 - 2,47	0,30 - 1,25	7,40 - 17,09	5,27 - 22,60	3.23 -19.00	5.50 – 15.00	0.54 - 3.60)	6.25 - 46.50	5,52 - 13,13	0,49 - 0,98	0,09-0,31	7.82 - 11.80	6.05 - 7.80	2,79 - 5,56

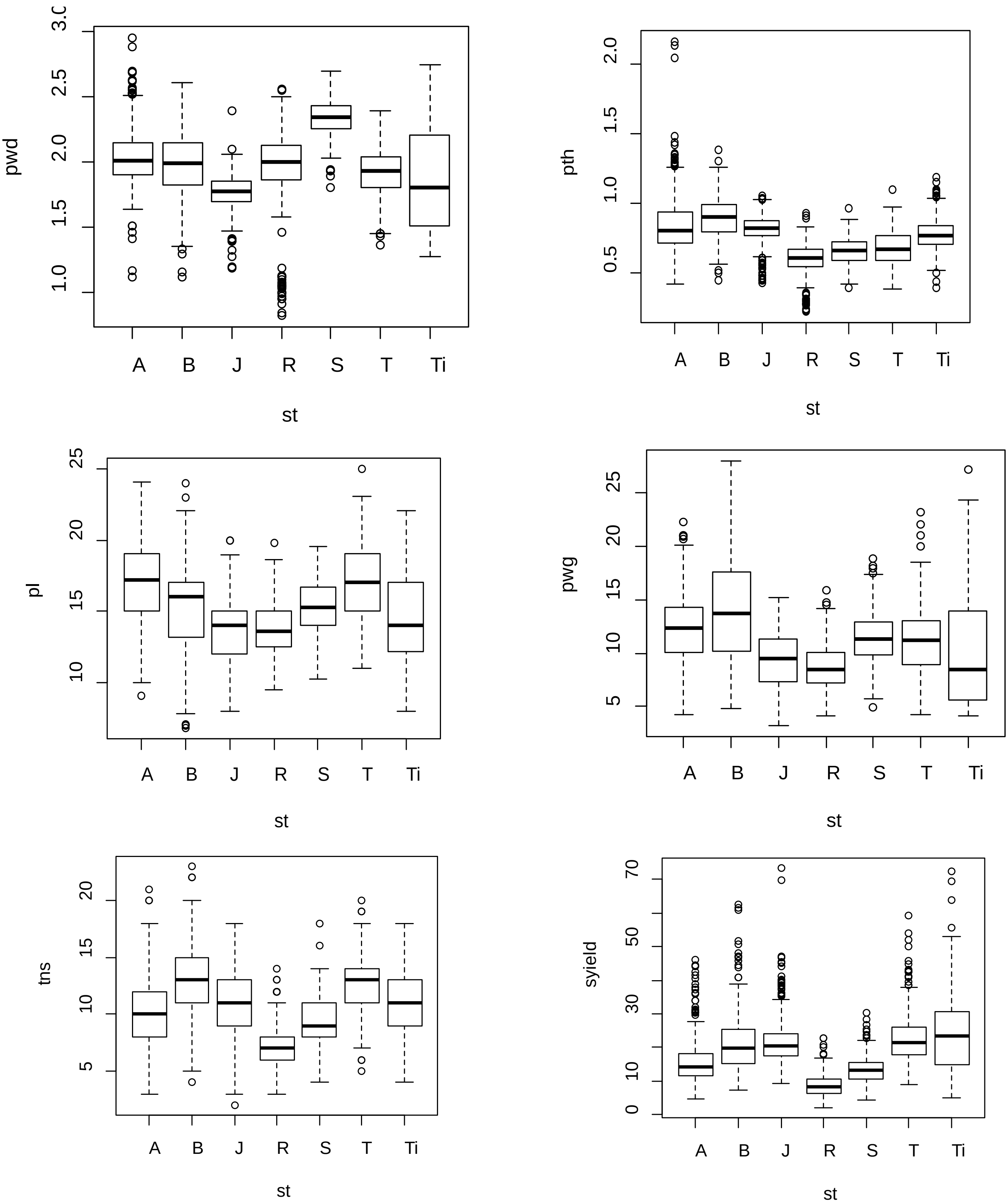

Figure 2: Boxplot of pod and seed traits per population.

Pod chord: This trait averaged 11.97 cm. The highest mean was registered in Tlemcen, followed by Blida, and the lowest one in Jijel. Between provenances variation of this trait was less pronounced comparatively to other pod traits.

In average, pod chord represented 79% of pod length (range per provenance: 73-84% and per tree: 49-98%). 71.43 % of individuals pertained to the intermediate class i.e.] 0.65 - 0.85]. At provenance level, 100% of individuals from Setif pertained to this class whereas 70% of those from Jijel pertained to the class] 0.85 - 1.00].

Ratio “pod length/pod width”

In average, pods were 7.82 times longer than wider (range per tree: 5.52 – 13.13 and per provenance: 6.57 - 8.94% for Setif and Tlemcen respectively).

As a whole, 65% of individuals pertained to the same class of ratio i.e.]7 - 10]. Relizane and Setif showed respectively 65 and 70% of individuals in the class of low ratio i.e.]5 - 7] which indicates their relatively larger pods.

Mean seed weight: In average, a seed weighed 0.17 g. The heaviest seeds were registered in Blida and the lightest ones in Relizane. The former provenance had 75% of individuals with mean seed weight ≥ 0.20 g and the latter 100% of individuals with mean seed weight < 0.15 g.

Seed length: Overall, seed length averaged 9.12 mm. The longest seeds were found in Annaba followed by Blida and the narrowest ones in Relizane. Annaba contained no individual in the class of short pods, contrary to Relizane which contained 100% of individuals in this class (Table 4/Fig. 3).

Seed width: Seed width averaged 6.84 mm. The largest seeds were observed in Tipaza followed by Setif and the thinnest ones in Relizane. Individuals with large seeds are missing in Relizane and those with narrow seeds are missing in Tipaza. The other provenances contained individuals pertaining to the 3 classes of this trait (Table 4).

Seed thickness: In average seeds are 4.04 mm thick. The thickest seeds were recorded in Blida and Jijel and the thinnest ones in Relizane. Indeed, 100% of individuals from Relizane pertained to the class of low mean values; 100% of those from Setif pertained to the class of high mean values. Individuals of the remaining provenances pertained mainly to the class of intermediate means.

As a whole, the ranking of provenances for mean values of pod and seed traits showed an inconsistent pattern according to the trait of interest. Relizane segregated from the other provenances by the lowest means for 9 traits of which 4 are related to seed size, 3 to pod size and 2 others to seed production (number of seeds per pod and seed yield). The only trait for which such provenance was ranked in an intermediate position is pod width which registered the lowest mean at Jijel. The top position for high mean values was occupied by some provenances (Annaba, Blida and Tlemcen) for 3 to 4 traits; by Tipaza for seed width and seed yield and by Setif for pod width. Conversely, the bottom position for the lowest mean values was occupied by Jijel just behind Relizane for 2 pod traits (length and weight) and 2 seed ones (length and width).

The two-way nested Anova revealed significant differences between provenances for all considered traits (Table 5). It also revealed slight but significant differences within-provenance, i.e. among trees. The extent of among provenance differences for pod and seed traits is the highest for seed thickness and the lowest for pod chord (Fig. 4).

Table 4: Distribution of trees within-provenance per class of mean values of pod and seed traits.

Trait	Class of mean values per tree	% of trees within-provenance (n=20)							% of trees (n=140)
		A	B	J	R	S	T	Ti	
Pod length (cm)	≤14	15	30	75	95	15	10	55	42,14
	]14-18]	40	45	25	5	85	55	35	41,43
	>18	45	25	0	0	0	35	10	16.43
		A	B	J	R	S	T	Ti	
Pod width (cm)	]1 - 1.5]	0	0	0	5	0	5	20	4.29
	]1.5 - 2]	70	65	100	50	0	65	45	56.43
	]2 - 2.5]	30	35	0	45	100	30	35	39.29
		A	B	J	R	S	T	Ti	
Pod thickness (cm)	]0.30 - 0.60]	0	0	5	30	0	25	0	8.57
	]0.60 - 0.90]	70	55	90	70	100	75	90	78.57
	> 0.90	30	45	5	0	0	0	10	12.86
		A	B	J	R	S	T	Ti	
Pod weight (g)	]5 - 10]	20	15	60	85	15	30	50	39.29
	]10 - 15]	80	50	40	15	85	65	25	51.43
	> 15	0	35	0	0	0	5	25	9.29
		A	B	J	R	S	T	Ti	
Total number of seeds	]5,5 - 8]	10	0	5	85	15	0	5	17,14
	]8 - 10,5]	45	0	35	15	70	0	25	27,14
	]10,5 - 13]	45	55	60	0	15	75	60	44,29
	>13	0	45	0	0	0	25	10	11,43
		A	B	J	R	S	T	Ti	
Seed yield (%)	≤10	0	0	0	80	0	0	0	11,43
	]10-15]	60	15	0	20	95	0	20	30
	]15-20]	30	30	30	0	5	30	25	21,43
	>20	10	55	70	0	0	70	55	37,14
		A	B	J	R	S	T	Ti	
Mean seed weight(g)	<0,15	0	0	0	100	0	0	0	14,29
	[0,15 -0,20[	90	20	85	0	100	0	0	42,14
	[0,20 -0,25[	10	55	10	0	0	60	70	29,29
	≥0,25	0	25	5	0	0	40	30	14,29
		A	B	J	R	S	T	Ti	
Seed thickness (mm)	]2.5-3.5]	0	0	0	20	0	0	0	14.29
	]3.5-4.5]	18	16	16	0	0	13	17	57.14
	> 4.5	2	4	4	0	20	7	3	28.57
		A	B	J	R	S	T	Ti	
Total seed weight (g)	≥1	0	0	0	20	0	0	0	14.29
	] 1-2]	18	0	12	0	20	1	12	45
	>2	2	20	8	0	0	19	8	40.71
		A	B	J	R	S	T	Ti	
Seed width (mm)	]6-6.5]	45	15	40	25	5	15	0	20.71
	]6.5-7]	20	50	50	75	50	40	55	48.57
	>7	35	35	10	0	45	45	45	30.71
		A	B	J	R	S	T	Ti	
Seed length (mm)	] 7-9]	0	10	70	100	75	50	45	50
	] 9-11]	95	85	30	0	25	50	50	47.86
	>11.00	5	5	0	0	0	0	5	2.14

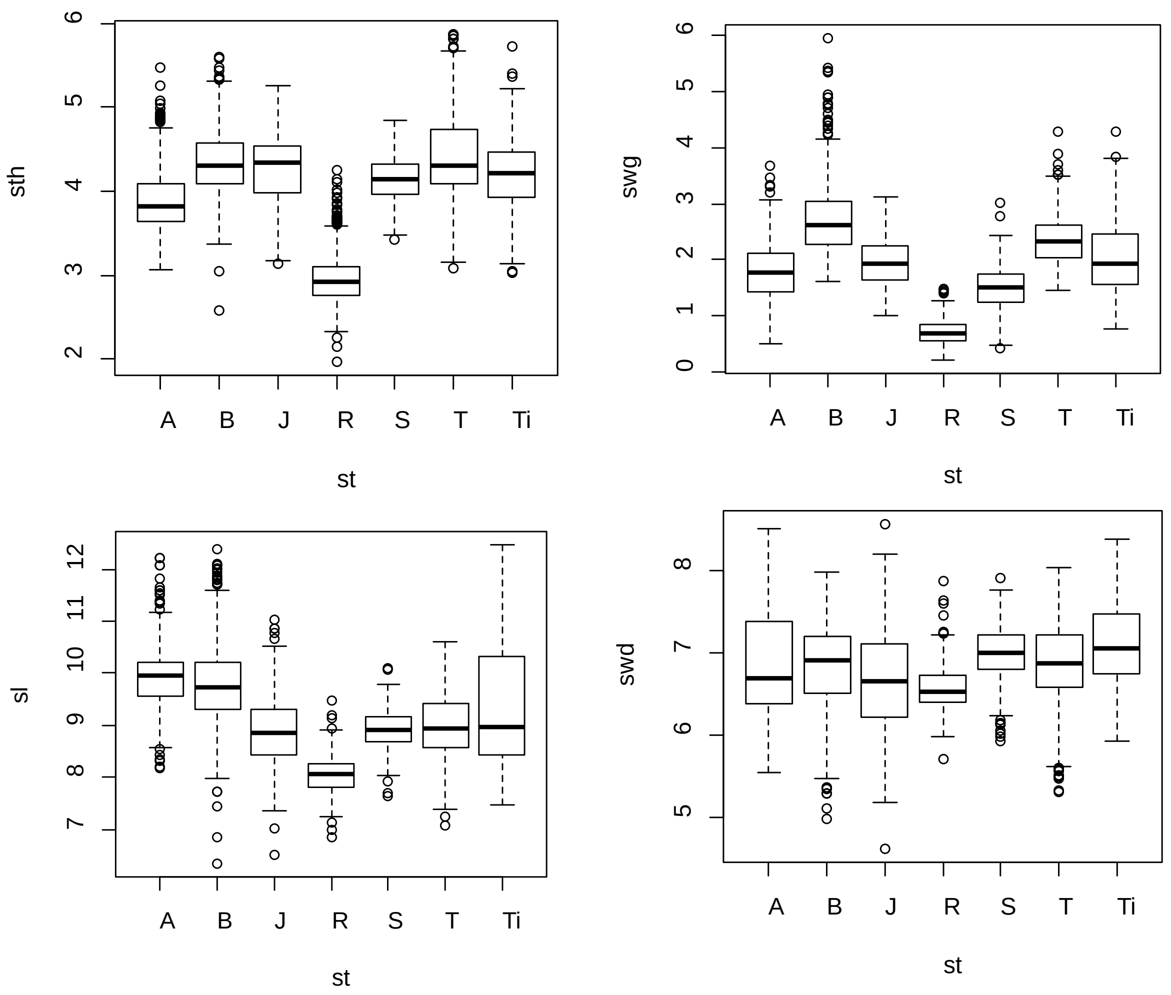

Figure 3 : Continued: Boxplot of pod and seed traits per population.

Table 5: Results of two way nested Anova on quantitative traits of pods and seeds.

Trait	**Source of variation**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Pod width	st	6	82.76	13.793	631.01	<2e-16 ***
	st:tree	133	102.79	0.773	35.36	<2e-16 ***
	Residuals	2660	58.14	0.022		
	st	6	28.72	4.786	504.83	<2e-16 ***
Pod thickness	st:tree	133	25.18	0.189	19.97	<2e-16 ***
	Residuals	2660	25.22	0.009		
Pod length	st	6	4540	756.6	271.94	<2e-16 ***
	st:tree	133	10320	77.6	27.89	<2e-16 ***
	Residuals	2660	7401	2.8		
Pod weight	st	6	8513	1418.8	235.87	<2e-16 ***
	st:tree	133	20489	154.1	25.61	<2e-16 ***
	Residuals	2660	16001	6.0		
Pod chord	st	6	577	96.20	27.83	<2e-16 ***
	st:tree	133	8394	63.11	18.26	<2e-16 ***
	Residuals	2660	9196	3.46		
Seed yield	st	6	79404	13234	392.93	<2e-16 ***
	st:tree	133	57944	436	12.94	<2e-16 ***
	Residuals	2660	89589	34		
Total number of seeds/pod	st	6	9187	1531.1	307.973	<2e-16 ***
	st:tree	133	4822	36.3	7.293	<2e-16 ***
	Residuals	2660	13224	5.0		
Mean seed weight	st	6	3.538	0.5897	345.844	
	st:tree	133	1.272	0.0096	5.609	<2e-16 ***
	Residuals	2660	4.536	0.0017		
Seed thickness	st	6	605.8	100.97	1218.4	<2e-16 ***
	st:tree	133	225.9	1.70	20.5	<2e-16 ***
	Residuals	2660	220.4	0.08		
Seed weight	st	6	1002.9	167.14	890.384	<2e-16 ***
	st:tree	133	201.9	1.52	8.086	<2e-16 ***
	Residuals	2660	499.3	0.19		
Seed length	st	6	929.9	154.98	701.42	<2e-16 ***
	st:tree	133	837.1	6.29	28.49	<2e-16 ***
	Residuals	2660	587.7	0.22		
Seed width	st	6	77.8	12.961	89.32	<2e-16 ***
	st:tree	133	303.0	2.278	15.70	<2e-16 ***
	Residuals	2660	386.0	0.145		

Significance code: '***' : '0.001'

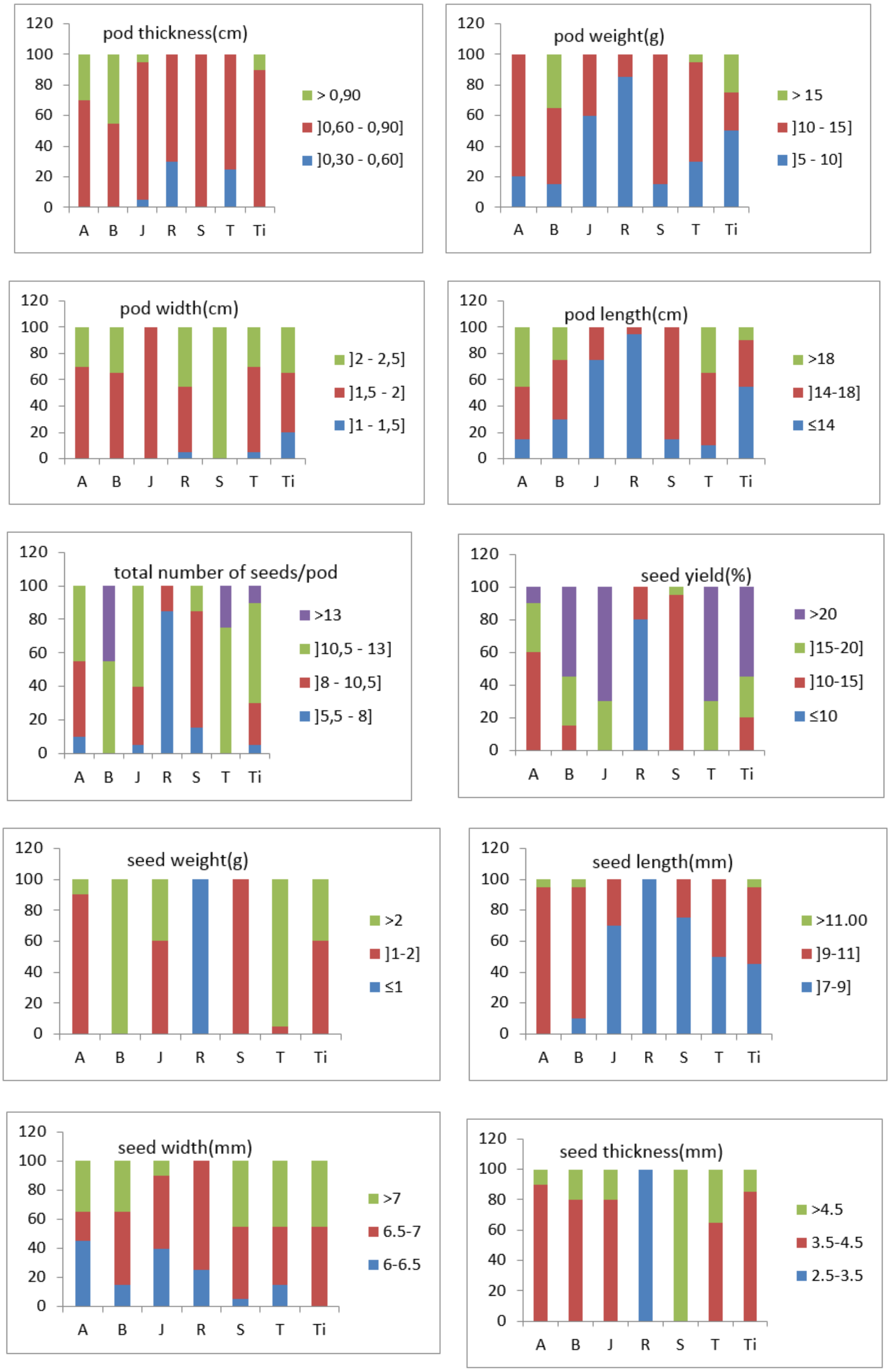

Figure 4 : Distribution of trees within-provenance for mean values of pod and seed traits

3.2.2. Correlation between quantitative traits

The strongest correlation was observed between pod weight and pulp weight (r = 0.98) indicating that pod weight is mainly determined by pulp weight (Table 6).

Among the 4 pod size traits (i.e. pod weight, length, width and thickness), pod weight revealed positive correlations with all other pod traits and with seed length.

Among the 4 seed size traits (i.e. mean seed weight; length; width and thickness), seed length showed positive correlations with all other seed traits and with all pod size traits except pod width.

Seed yield revealed slight but positive correlations with seed weight (r = 0.38) and seed thickness (r = 0.38) and negative correlations with pulp weight (r = - 0.54); pod weight (r = 0.42) and pod width (r = - 0.42).

Table 6 : Correlation matrix between quantitative traits of pods and seeds

	pl	pwd	pth	chr	pwg	tns	mswg	sl	swd	sth	tswg	ppwg	syield
pl	1												
pwd		1											
pth			1										
chr	0,58			1									
pwg	0,63	0,45	0,43	0,49	1								
tns					0,30	1							
mswg			0,30				1						
sl	0,37		0,37		0,42	0,31	0,39	1					
swd								0,36	1				
sth			0,35			0,40	0,42			1			
tswg	0,31		0,38			0,78	0,64	0,50		0,54	1		
ppwg	0,61	0,48	0,38	0,48	0,98			0,35				1	
syield		-0,42			-0,42	0,49	0,38			0,38	0,57	-0,57	1

Correlation coefficients lower than 0.30 are not shown. All correlation coefficients are significant at α = 0.001(n = 2800).

3.2.3. Correspondence between quantitative and qualitative traits

The examination of a possible correspondence between pod and seed qualitative traits indicated a variable pattern with only a few cases of concordance between modalities of traits. For example, the dark-brown color was dominant both among pods and seeds of Jijel and the light brown color both among pods and seeds of Relizane, contrary to Tlemcen and Blida where the dark brown-color was dominant on pods and the light- brown color on seeds. Setif and Tipaza were the only provenances which had pods and seeds of similar surface (i.e. smooth).

Qualitative pod traits did not seem to be linked to quantitative pod ones, suggesting for example, that pod color at tree level may not be a good predictor of seed yield. Indeed, this agronomic trait ranged between 10.40 and 30.53% ; 10.18 and 38.32% ; 6.25 and 21.60% among trees bearing 100% reddish-brown; dark-brown and light-brown pods respectively. This applies also to the relationship between aspect of pod surface and seed yield.

In Tipaza for example, trees bearing pods of 100% smooth surface showed either high i.e. 46.5% or low i.e. 13.70% seed yield. Similarly, in Relizane seed yield was invariably low both among trees with 100% smooth pod surface and those with 100% rough pod surface.

DISCUSSION AND CONCLUSION

The morphological analysis of carob pod and seed quantitative traits revealed significant variations between and within-populations. Qualitative traits (color, shape and surface aspect of pods and seeds) also proved variable within and between populations.

3 pod colors were registered as a whole: Light-brown, dark-brown and reddish brown in agreement with data literature on the species [52, 9, 2, 46]. The dominant pod color varied according to provenance: blackish-brown in Tlemcen, Blida and Jijel; light-brown in Relizane and reddish-brown in Tipaza and Setif.

3 modalities of pod shapes were registered: straight; curved and spiral, in concordance with literature [9, 46], but the curved shape was dominant across all provenances.

Pod surface varied from smooth to wrinkled in concordance with other authors [9, 46]. The wrinkled surface was better represented among 5 provenances and the smooth surface in one provenance. Among the 4 modalities of seed color recorded, the light-brown was the most represented across all provenances except Annaba and Jijel which rather showed a higher proportion of dark-brown seeds.

Among the three modalities of seed surface recorded, the dominant one corresponded to the smooth surface in agreement with other sources [4, 9, 46].

Among the three modalities of seed shapes registered, the rounded shape concerned half of the seeds and was represented at very high rates in Setif and Relizane. Oval seed shapes were dominant in Blida and elliptical ones dominated in Tipaza.

Regarding the quantitative traits of pods and seeds, the extent of their variation within and between population depended on the trait of interest: the least pronounced between-provenance variation was registered for pod chord and the most pronounced one for seed thickness.

The rank of provenances for their mean values depended on the trait of interest: Relizane cumulated the lowest mean values for 9 quantitative pod and seed traits; followed by Jijel for 4 traits; while the top position for high mean values was occupied by 3 provenances (Annaba, Blida and Tlemcen) for 3 to 4 traits, Tipaza for seed width and seed yield and by Setif for pod width.

Comparatively to authors' findings, overall means registered for pod size proved either similar [3, 18] in the case of pod weight ; lower [8, 54,60,11, 45, 18] in the case of pod length or even higher [28, 23,19].

The population range for pod traits proved larger than those reported by one source [59] and overlapping with those mentioned by other sources [9, 7, 35, 46, 38, 29].

The overall mean registered on total seed weight per pod lies within the range reported for carob[19, 28, 11, 8]. The population range for this trait is larger than that found by Konaté et *al.* [35] and it overlaps with that registered by Haddarah et *al.* [29].

Seed dimensions (i.e. length, width and thickness) displayed overall means which are also lie within the range of values reported for carob [54, 28,23,19,11].

Indeed, the lowest means displayed by Relizane population for traits such as seed thickness, length, seed yield and mean seed weight lie within those reported in the Berkane ecoregion in Northern Morocco [59].

Comparatively to the authors findings[61, 46, 59], range per population for these traits proved either lower or similar. Mean seed weight proved slightly lower than other Authors' findings [3, 64, 18, 8]. The population range for this trait overlaps with that given by other authors [7, 61, 59, 45, 38 , 61] reports that a seed weight of 0.20g is the minimum required for a cultivar to be considered of medium quality for industrial applications. In the present study only the Blida population reached such threshold with an average of 0.23g.

Overall, seed yield proved higher than that of European cultivars[3, 8] and lower than that of Moroccan samples [28, 19]. The population range proved comparable to that reported by Naghmouchi et *al.* [45] and overlaps with that reported by other authors [31, 61, 7, 10, 59, 38, 17]. At population level, Tipaza registered the highest seed yield followed by Blida and Tlemcen which contained respectively 50% and 70% of individuals with seed yield >20%. Such individuals may be promising as a potential source of raw material.

Regarding correlations between traits, contrary to pod thickness and seed width, pod weight proved to be the most correlated trait to both other pod traits and seed ones. Correlations between pod weight and other pod and seed traits are concordant with those mentioned by Bostan & Kaşko Arici [11]. Those between pod length and other pod traits are concordant with [59].

The high positive correlation between pod weight and pulp weight ($r = 0.98$) indicates that the heavier the pods the higher may be their pulp content. Conversely, pod weight was negatively correlated to seed yield in agreement with [4, 7, 45] but the strength of such relationship is higher according to Naghmouchi et *al.* [45].

Correlations between the total number of seeds per pod and pod size traits are weak. This trend is concordant with Carlson [13] who observed no apparent relationship between the number of kernels per pod and the size of pod.

The examination of a possible concordance between qualitative and quantitative traits at tree and provenance level did not show any particular trends. For example, pod color, an easily measurable trait, did not show a particular concordance with seed yield. That is, similar ranges of seed yield were observed among trees which produced either 100% of clear; reddish or blackish brown pods. On the other hand, only in a few cases were concordances between qualitative attributes of pods and seeds signaled.

The geographic range explored in this study, corresponds to the northern fringe of carob in North Africa. Moroccan carob has been studied both in the same fringe [35, 59] and more Southward in the high Atlas regions [59]. Some Tunisian sampling sites [18,45] extend more Southward than Tlemcen which represents the southernmost sampled site in our study but not the southernmost one in Algeria.Indeed, carob populations are also found more southerward in sites such as El-Bayadh, though in marginal situations, probably due to their degradation [40].

Despite the large geographic area presently explored, from East to West, results indicated a pattern of variation not clearly related to bioclimate, elevation or distance from the sea coast. Relizane (an inland population located at an elevation of 155 m within a mild semi arid bioclimate) segregated from the other populations by the lowest means (for 9 quantitative traits) followed by Jijel for 4 traits (a coastal population of eastern Algeria, located at an elevation of 81 m and evolving under a mild humid bioclimate) and then by Tipaza for 2 traits (pod length and pod weight). On the other hand, Blida (a north-central provenance located at an elevation of 187 m in a warm subhumid bioclimate) shared high mean values of seed length, pod weight and pod thickness with Annaba (a coastal population of eastern Algeria located at 740 m and evolving under a mild humid bioclimate).Blida also shared high mean values of seed thickness; mean seed weight and total seed weight with Tlemcen (a Western population located at 757 m and evolving under a mild semi-arid bioclimate). The two most distant populations, i.e. Annaba and Tlemcen, shared high means of pod length. The two coastal provenances of eastern Algeria (Jijel and Annaba) were ranked each with a provenance from either North central or North-western Algeria. The coastal provenances (Annaba, Jijel and Tipaza) and the inland ones (Tlemcen, Relizane and Setif) showed a segregation pattern that did not totally match their distance from the sea coast.

The absence of concordance between ecological conditions (bioclimate, elevation and distance from the sea coast) at the site of origin and the variation pattern of carob pod and seed morphology is in discordance with the general trend, under natural conditions, where there is a close relationship between the morphological and the physiological traits of plants and of habitats in which the traits have evolved and are expressed [51], The observed trend is also in discordance with authors who carried out similar studies on carob. El Ferchichi et *al.* [18] and Naghmouchi et *al.* [45] suggested edapho-climatic factors as a source of variation observed among Tunisian populations. Environmental factors have also been suggested as a possible source of carob pod and seed traits variation among Moroccan populations by Gharnit et *al.* [28] and Sidina et *al.* [59] who found highest pod traits among Northern ecoregions of Morocco comparatively to southern ones. Similarly, Haddarah et *al.* [29] observed a latitudinal gradient among Lebanese varieties of carob for pod and seed traits with seed mass in the field positively correlated with latitude and longitude.

The present study of carob populations is based on fruits and seeds which are components of plant sexual reproduction and traits such as seed number per fruit and seed weight may be subjected to plant adjustment [48, 58] as may also be adjusted other reproductive parameters such as the number of fruits per plant as a consequence of predation [5] and resource limitation [58]. Fruit production may also fluctuate from year to year as a result of alternation, a phenomenon largely acknowledged in perennial plants [33, 34]. Such alternation may result in fluctuation of reproductive parameters such as the number of cones per tree and of seeds per cone [36] on Atlas cedar and seed weight on *Nothofagus nervos* [41] and carob [13, 61, 32]. Therefore, it may be interesting to test the stability of the variation pattern presently observed.

On the other hand, in the perspective of identifying the best genotypes for breeding programs and/or accession collection, additional descriptors should be examined such as regular bearing [13,61, 32]; gender and harvest period or fruit ripening [13,61], pedicel length as a criterion of pod abscission [61]; trunk cross section and canopy volume [61], fruit sweetness, flowering and fruiting phenology and precocity [37].

The study showed consistent variation among wild carob populations for pod and seed morphology even though such variation did not seem to be geographically structured. The analyzed traits may be considered under an agronomic and an industrial perspective (seed yield and pulp weight) and/or an ecological one (seed size and number are components of plant sexual reproduction that may have implication on its regeneration potential). Such descriptors, together with additional ones, will contribute to the characterization of carob genetic resources and guide the choice of populations for *in situ* conservation or as a source of material for restoration; breeding programs and germplasm collections.

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