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## Genetic Variability of Wild Provenances of *Acacia tortilis* ssp. *raddiana* (Savi) Brenan in South of Morocco

1.2 Fatima El Ayadi, <sup>1</sup>Naima Ait Aabd, <sup>1</sup>Aissam El Finti, <sup>1</sup>Fouad Msanda,
 <sup>2</sup>Fouzia Baniaameur and <sup>1</sup>Abdelhamid El Mousadik
 <sup>1</sup>Laboratory Biotechnology and Valorization of Natural Resources,
 Faculty of Science, B.P. 8106, Agadir, Morocco
 <sup>2</sup>Laboratory Research on Genetics Variability, Bioinformatics and Mathematics of the Living,
 Faculty of Science, B.P.8106, Agadir, Morocco

Abstract: In this study, variability in eight pod traits of 300 genotypes (mother-tree) of *A. tortilis* ssp. *raddiana* (Savi) Brenan collected from southern regions of Morocco was assessed. In the analysis of variance, we found that *Acacia raddiana* have significant differences in all traits due to genotype within provenances, but only in pod length, seed weight per pod, seed number per pod, infected seed number per pod and 100-seed weight due to provenances. In general phenotypic coefficient of variation was higher than the genotypic coefficient of variation, indicating the predominant role of environment. High heritability and genotypic gain were recorded for 100-seed weight (92.75 and 17.20%), empty pod weight (53.68 and 21.18%) and pod weight (46.45 and 16.13%), respectively, indicating the additive gene action. Seed weight had positive correlation with seed length, pod weight, seed weight per pod and empty pod weight. On the basis of the Principal Component Analysis (PCA) and the hierarchical cluster analysis, respectively, tow clusters were obtained with highest number of provenances falling under cluster I. The first cluster is composed of the provenances: Agdz, N'koub, Tazarine, Mceissi, Tata and Tissint, the second cluster presented by the provenances: Foum Zguid, Gluemim, Mseid and Assa. Among the clusters formed cluster 1 presents the best means for the majority of traits studied.

Key words: South of Morocco, Acacia raddiana, biometry, morphological diversity, Pods, variation

#### INTRODUCTION

Acacia is the second largest genus in the family Leguminosae with about 1350 species (Maslin, 2003; Maslin et al., 2003). The current classification of Acacia differentiates three subgenera (Ross, 1979; Maslin et al., 2003): Acacia, Heterophylum and Aculeiferum, Acacia raddiana belongs to the acacia subgenus. The base chromosome number in the genus Acacia is x = 13 with polyploidy occurring in several species, (Bukhari, 1997; Blakesley et al., 2002; Khatoon and Ali, 2006). In the south of Morocco who is the most arid region, larger part of the area is an absolute desert with almost no vegetation and one can easily travel for large area without coming across a single plant. In contrast to this harshness, a great number of prehistoric agreement remains provide evidence for rich environmental resources in the past. Many studies of pollen and wood fossils concluded that the vegetation of the coastal strip should be very close to the current while inside grew a steppe of Acacia raddiana (Barathon and Riser, 1999). Acacia tortilis (Forssk.)

Hayan, member of the flora xero-tropical Africa, was spread abundantly in all African countries, extends eastward to the Arabian Peninsula and Southwest Asia (Al-Mefarrej and Elkhalifa, 2006) during the early and middle Paleocene which may have coincided with a savanna-like environment (Maley, 1980). Based on current information, we admit that this taxon is present in the saharian and sub saharian African countries and in the Middle East (Le Floc'h and Grouzis, 2003). With desertification and ecosystem degradation problems in mediterranean regions (Romdhane et al., 2006) Acacia tortilis (Forsk.) Hayne ssp. raddiana (Savi) Brenan (Family Fabaceae; subfamily Mimosoideae) (Brenan, 1957) remains one of the only wild Acacia, which grow spontaneously in arid and Saharian areas which makes it an important reforestation species. It is characterized by a large ecological plasticity, colonizing regions receiving between 50 and 1,000 mm of annual precipitation (El Ferchichiet et al., 2009). It plays an important role for fertility of poor and eroded soils, this is proved by studies on the effect of Acacia trees on their physical, chemical,

and biological environments in different ecosystems (Hagos and Smit, 2005; Abule et al., 2005; Burke, 2006), the positive influence of Acacia tortilis subsp. raddiana in terms of soil enrichment in arid ecosystems is conformed especially for to soil organic matter, total N, P and Ca2+ (Abdallah et al., 2008). Also, the study on the relationships between the morphological and the environmental variables of Acacia tortilis, showed that the last one contribute to the expression of morphological variable expressed by the species (Abdelrahman and Krzywinski, 2008). The savanna of Acacia raddiana is under two constraints: tree mortality (Ward and Rohner, 1997; Shrestha et al., 2003) and infestation of seeds. The degree of seed parasitism varied strongly between trees and years (Derbel et al., 2007). In Morocco the specie of A. tortiliss sp. tortilis (Forsk.) is represented by subspecies, Hayne, A. tortilis ssp. raddiana (Savi) Brenan (Mhirit and Blerot, 1999). Regeneration of the trees is made difficult owing to overgrazing, which adds its limiting effects to those of aridity (Bijaber and Ahlafi, 2005) and infestation rate. In this context, the preservation and sustainable management of genetic resources of this millennium species is proved necessary for maintaining the biodiversity of its ecosystem. Morphological variation in seed and pod traits among the natural populations of species is useful in selection programmes for genetic improvement (Bahadur and Hooda, 1995). The morphological variability signifies the adaptation of the species to the environment and it may be genetically determined or environmentally induced (Chiveu et al., 2009).

Limited work has been done on *Acacia raddiana* in south of Morocco, with absolutely no research on its genetic variability basis on morpho-metric traits, however, the variation, if present, have practical implications because reforestation programs collect seed from provenances where genotype presents good quality of traits. In the present study, we have evaluated the relative magnitude of variation in pod traits within and among provenances of *A. tortilis* ssp. *raddiana* (Savi) Brenan in south of Morocco and estimate the genetic parameters under different ecological conditions.

### MATERIALS AND METHODS

Presentation of plant materiel and site of study: Genotypes samples were collected from ten isolated natural provenances Agdz, N'koub, Tazarine, Mceissi, Tata, Tissint, Foum Zguid, Gluemim, Mseid and Assa (Fig. 1), which were chosen to represent a range of different geographical locations between latitude 28°02' N

Table 1: Description of pod collection sites

Province	Provenance	Altitude (m)	Latitude	Longitude
Zagora	Agdz (1)	834	30°45' N	5°30' W
	N'kob (2)	960	30°50' N	5°44' W
	Tazarine (3)	1100	30°42' N	6°31' W
	Foum Zguid (4)	743	30°13' N	6°51' W
Errachidia	Mecissi (5)	820	31°13' N	4°48' W
Tata	Tissint (6)	676	29°51' N	7°30' W
	Tata (7)	630	29°43' N	7°57' W
Guelmim	Guelmim (8)	527	28°48' N	9°27' W
Tan Tan	Msied (9)	249	28°02′ N	10°50 W
Assa-Zag	Assa (10)	385	28°42' N	9°14' W

to 31°13' N and longitude between 4°48' W to 10°50 W in south of Morocco (Table 1).

Three hundred trees were identified based on the eight morpho-metric traits of mature pods collected during June 2009, from different sites: pod length (LP) (cm), pod width (WP) (mm), pod weight (WtP) (g), seed weight per pod (WtS/P) (g), empty pod weight (WtEpP) (g), seed number per pod (SN/P), Infected seed number per pod (InSN/P) and 100-seed weight (100-Wt) (g). Each provenance consisted of ten genotypes (mother-tree) and thirty pods per tree with three replications to provide genetic variation.

Seed is considered infected if there is a visibly open hole, still closed, or when it is dark (Fig. 2).

Statistics analysis: A general linear model (ANOVA) analysis was used to determine the effect of site and genotype on all morpho-metric traits. Provenance effects were considered fixed because they were not randomly chosen. Genotypes (mother-trees) were treated as random effects. Factor mother-tree was hierarchical to the factor provenance because the genotypes were not repeated between sites. Data were not transformed, even little of variables had a non-normal distribution. Analysis of variance is relatively robust against departures from normality, of greater concern is heterogeneous variance among experimental groups (Glass *et al.*, 1972). All statistical analyses were performed with Statistical V.6.

Analysis of variance was carried out on data following hierarchical model (Nanson, 1970) (Eq. 1):

$$Y_{iik} = \mu + P_i + G_{i(i)} + \varepsilon_{iik}$$
 (1)

where,  $Y_{ijk}$ ,  $\mu$ ,  $P_i$ ,  $G_{j(i)}$  and  $\epsilon_{ijk}$  represent, respectively, the observed value for an individual, the provenance mean, the effect of provenance, genotype in provenance and the experimental error. The factors P (provenance) and G (genotype) were defined successively fixed and random effect.

Differences between mean values were compared using the Duncan multiple range test (5%).

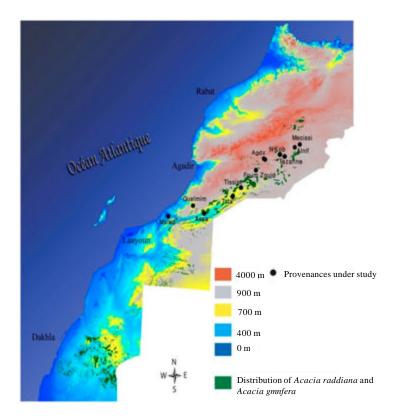


Fig. 1: Geographic localization of Acacia raddiana provenances under study



Fig. 2: Infected seeds of Acacia tortilis subsp. raddiana

**Genetic parameters:** The mean squares were used to estimate genotypic and phenotypic variance according to Johnson *et al.* (1955). Phenotypic (PCV) and genotypic (GCV) coefficients of variation were calculated as the following:

$$PCV = (\sigma p / \overline{x}) \times 100$$
 (2)

$$GCV = (\sigma g / \overline{x}) \times 100$$
 (3)

where,  $\sigma_p$ ,  $\sigma_g$  and  $\bar{x}$  are the phenotypic, genotypic standard deviations and mean across location, respectively (Baye and Becker, 2005; Diaz *et al.*, 2007).

Heritability in the broad sense (h²) (Eq. 4) was estimated on genotypic mean basis as described by Nanson (1970) and Jacques *et al.* (2004)

$$h^2 = \sigma_G^2 / \sigma_P^2 \tag{4}$$

where,  $\sigma_g^2$  and  $\sigma_p^2$  are the genotypic and phenotypic variances, respectively.

Conventional genotypic gain was estimated following the equation (Eq. 5) (Nanson, 2004; Jacques *et al.*, 2004)

$$\Delta G_c\% = h_2.CVP$$
 (5)

**Multivariate analysis:** The Principal Component Analysis (PCA) was performed on the mean values of measured traits presented significant differences for each provenance, followed by Hierarchical classification witch leads to aggregate and store the provenances into classes according to the "distance" between them. This

Table 2: Descriptive statistics of characters measured in 100 genotypes within 10 provenances of Acacia tortilis (Forsk.) Hayne ssp. raddiana (Savi) Brenan [mean character values, degree of freedom (Df), standard deviation (SD), coefficients of variation (%), R<sup>2</sup> (%) intensity effect and the associated F for the significance of the differences]

		Prove	Provenances					Genotype/Provenance				
Variables	Mean	$\mathbf{Df}$	(SD)	CV %	F	$\mathbb{R}^2$	Df	(SD)	CV %	F	$\mathbb{R}^2$	
Pod length	13.16	9	3.54	26.88	3.27**	24.64	90	3.54	26.88	22.37***	75.36	
Pod width	7.47	9	0.91	12.17	$0.47 \mathrm{ns}$	4.54	90	0.91	12.17	62.38***	95.46	
Pod weight	1.11	9	0.39	34.70	1.08*	9.77	90	0.39	34.70	27.02***	90.23	
Seed weight/pod	0.47	9	0.18	37.91	2.31*	18.77	90	0.18	37.91	18.58***	81.23	
Empty pod weight	0.65	9	0.25	39.12	0.75*	7.01	90	0.25	39.12	35.76***	92.99	
Seed number/pod	7.68	9	2.14	27.87	3.98***	28.50	90	2.14	27.87	8.58***	71.50	
Infected seed number/pod	3.71	9	3.23	87.16	31.68***	76.01	90	3.23	87.16	12.45***	23.99	
100-seed weight	6.44	9	1.18	18.39	$0.90\mathrm{ns}$	8.30	90	1.18	18.39	385.06***	91.70	

<sup>\*\*\*</sup>Significant at  $\alpha$ = 0.001; \*\*Significant at  $\alpha$  = 0.01; \*Significant at  $\alpha$  = 0.05; ns: Non-significant

Table 3: Mean Performance of 10 provenances of Acacia tortilis (Forsk.) Hayne ssp. raddiana (Savi) Brenan for different traits

						() —				
Variables	Agdz	N'kob	Tazarine	F.Zguid	Mecissi	Tissint	Tata	Gluemim	Msied	Assa
Pod length	13.07cd	13.80bc	13.91bc	11.92d	12.16d	15.68a	14.52ab	12.18d	11.91d	12.42d
Pod width	7.49a	7.35a	7.32a	7.77a	7.68a	7.38a	7.53a	7.27a	7.31a	7.51a
Pod weight	1.01b	1.23a	1.20a	0.97c	1.13ab	1.22a	1.14ab	1.06b	1.09b	1.06b
Seed weight/pod	0.46ab	0.53a	0.51a	0.37c	0.51a	0.50ab	0.49ab	0.41b	0.42b	0.43b
Empty pod weight	0.54c	0.69a	0.68a	0.59ab	0.61ab	0.71a	0.64a	0.64a	0.66a	0.63a
Seed number/pod	7.07cd	8.41a	8.01ab	6.41d	7.99ab	8.43a	7.64abc	7.30abc	7.96ab	7.52abc
Infected seed number/pod	0.37a	0.56a	1.11a	4.15bc	3.61b	6.67d	5.25c	4.56bc	4.09bc	6.70d
100-seed weight	6.93a	6.55a	6.69a	6.23a	6.75a	6.27a	6.66a	6.15a	5.76a	6.38a

Table 4: Spearman correlation among different morph-metrics traits of Acacia tortilis (Forsk.) Hayne ssp. raddiana (Savi) Brenan

	LP (cm)	WP (mm)	WtP (g)	WtS/P(g)	WtEpP (g)	SN/P	InSN/P
WP (mm)	0.155***						
WtP (g)	0.724***	0.475***					
WtS/P (g)	0.613***	0.381***	0.853***				
WtEpP (g)	0.677***	0.459***	0.930***	0.602***			
SN/P	0.668***	0.050	0.699***	0.707***	0.572***		
InSN/P	0.241***	0.010	0.145***	0.010	0.215***	0.278	
Wt100 <sub>unfS</sub> (g)	0.197***	0.471***	0.395***	0.504***	0.251***	0.049	-0.150

<sup>\*\*\*</sup>Significant at  $\alpha = 0.001$ . LP: Pod length; WP: Pod width; WtP: Pod weight; WtS/P: Seeds weight per pod WtEpP: Empty pod weight; SN/P: Seed number per pod; InSN/P: Infected seed number per pod Wt100: 100-seed weight

classification is realized on the number of axes considered attractive in the analysis of PCA.

#### RESULTS

**Analysis of variance:** For provenance and genotype, the morphological traits analyzed showed coefficients of variation (CV) ranging from 87.06% for infected seed number per pod to 12.18% for pod width (Table 2).

Effect of individual genotype within provenance was significant for all traits (Table 2)  $\alpha = 0.0001$ , but provenance was significant only for pod length, pod weight, seed weight/pod, empty pod weight, seed number per pod and infected seed number per pod. Comparison of performance means (Table 3) reveals that the provenances Tissint, Tazaraine and N'Koub showed the highest values for genotype traits. Except for infected seed number per pod, the highest mean values were noted within the provenance Tissint (6.67) and Assa (6.70), against provenances Agdaz, N'Koub and Tazaraine which show low means successively 0.37, 0.56 and 1.11.

Seed weight had positive correlation with seed length, pod weight, seed weight per pod and empty pod

weight. Weight of pods had a positive and high correlation with weight of empty pod (r=0.92). At additionally, positive and significant correlation with weight of seed per pods was shown r=0.84 and length of pods r=0.74 (Table 4).

Comparison of seed infestation among provenances (Table 3), shows that the site of Agdaz, N'Koub and Tazaraine have the lowest average (0.37, 0.56 and 1.11), while the highly average is noted for the provenance Assa 6.70 followed by Tissint 6.67. Infestations are caused mainly by *Bruchidius* sp. (Fig. 3a) and other insects (Fig. 3b, c).

**Provenances classification:** Hierarchical cluster analysis leads to identify tow major clusters. There are six provenances: Agdz, N'koub, Tazarine, Mceissi, Tata and Tissint in the first cluster, Foum Zguid, Gluemim, Mseid, and Assa are in the second cluster.

Genetic parameters: Genotypic and phenotypic variation, heritability and genetic gain: Variances of components for diverse traits are presented in Table 5. The magnitude of Phenotypic Coefficient of Variability







Fig. 3: (a-c) Insects infecting the seed of Acacia tortillis subsp. Raddiana

Table 5: Estimation of genetic variables for pods traits in Acacia tortilis (Forsk.) Hayne ssp. raddiana (Savi) Brenan

	Variance		Coefficient of variance (%)						
Variables	Genotypic	Phenotypic	Genotypic	Phenotypic	Heritability (%)	Genotipic advance (%)			
Pod length	4.80	11.54	16.65	25.81	41.61	10.74			
Pod width	0.58	0.86	10.18	12.42	67.17	8.34			
Pod weight	0.07	0.15	23.66	34.71	46.45	16.13			
Seed weight/pod	0.01	0.03	22.57	37.13	36.96	13.72			
Empty pod weight	0.03	0.06	28.91	39.47	53.68	21.18			
Seed number/pod	0.87	4.32	12.17	27.07	20.19	5.47			
Infected seed number/pod	1.58	5.72	33.89	64.48	27.63	17.81			
100-seed weight	1.32	1.43	17.86	18.55	92.75	17.20			

(PCV) was higher than the corresponding Genotypic Coefficient of Variability (GCV) for all characters. Infected seed number registered highest GCV (33.89%) and PCV (64.48%) followed by empty pod weight GCV (28.91%) and PCV (39.47%). Heritability estimates in broad sense were higher (92.75%) for 100-seed weight pod width (67.17%) and empty pod weight (53.68%). The genotypic advance as per cent of mean was ranged from 5.47% for seed number per pod to 21.18% for empty pod weight.

#### DISCUSSION

To manage genotype and environment interactions, the best genotype was selected for specific provenance to maximize deployment gains. This would involve identifying different provenances representing homogenous environmental zones within which selections are deployed and used in further breeding. The study of pod morpho-metrics traits of the natural populations is often considered to be useful step in the study of the genetic variability (Kaushilk et al., 2007). The proportion of pod walls is considered as a potential selection criterion for seed (Lagunes-Espinoza et al., 2000).

Variation, provenance performance and classification: Large significant ( $\alpha = 0.0001$ ) variation recorded for pod trait due to genotype within provenances in all traits was observed, with variation significant only for pod length, seed weight per pod, seed number per pod and infected seed number per pod among provenances. This result is consistent with the results of isozyme analysis of Senegalese stand and other African distributions, where Nei's genetic diversity is estimated at He in the range from 0.42 to 0.49 with a low differentiation between populations (Borgel et al., 2003) and those of the RAPD markers executed on populations of Acacia raddiana in the Nagev desert (Shrestha et al., 2002), however, contradict El Ferchichi et al. (2009), which looking an analysis diversity on four Tunisian populations of Acacia raddiana basis on there morphological characters concluded to high levels of variation of Acacia raddiana among provenances for the traits pod width, seed weight and seed number. According to these data, Acacia raddiana follow the model of Argania spinosa defined as generalist species (Bani-Aameur and Ferradous, 2001). The maintenance of this genetic diversity is partly explained by the polyploidy of these species. Variation was greatest for empty pods, weight seed per pod and number of seed infected per pod, which could be important criteria in selecting and classifying seed provenance.

Provenances Agdz, N'koub, Tazarine, Mceissi, Tata and Tissint, present the best means for the majority of

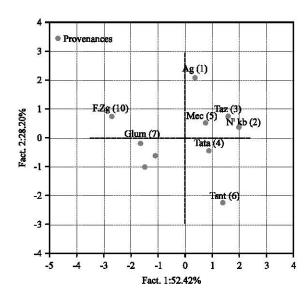


Fig. 4: Principal component analysis of ten *Acacia* raddiana provenances sampled

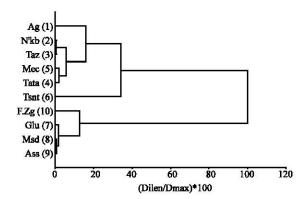


Fig. 5: Dendrogram (hierarchical clustering) of ten provenances of *Acacia raddiana* based on morpho-metrics traits

traits studied, which is strengthened with the results of principal component analysis (Fig. 4) and clearly in the dendrogram (hierarchical clustering) (Fig. 5) of the ten provenances. These sites are located in high altitude with low rainfall offset by the rivers and high temperatures during the summer season, unlike the provenance F. Zguid, where sampling is done on bare soil "Reg" or sand, pods was characterized with small and low seeds filling seed weight = 0.37 g. In Tunisia the best traits were determined in a population characterized by low rainfall and high temperatures (El Ferchichi *et al.*, 2009).

Variation of traits in *Acacia raddiana* pods could be due to the fact that the species grow over a wide range of

rainfall, temperature and soil type. Membrives et al. (2003) reported that some morphological characteristics evolve associated with soil texture. Raddad (2007) noticed that in Sudan, Clay plain provenances showed considerable variation in seed weight and seed number of pods of Acacia senegal. They had the smallest seed weight but the highest seed number, while the sand provenances had the largest seed weight but lowest seed number. Conversely, Acacia raddiana presents significant infestation rate. Seed infestation varied strongly between years for each tree and between trees for each year. Much of infestation already occurs on tree (Wiegand et al., 2000), from the beginning of pods formation (Derbel et al., 2007), where a majority of produced seeds were infested. Thus, the seeds have little chance of germinating; this follows the loss of natural regeneration of Acacia raddiana.

Genetic parameters: Genotypic variation is useful when selecting genotypes to improve particular traits (Okeno, 2002). Large difference between phenotypic and genotypic coefficient of variation is noted in all traits except for pod width and 100-seed weight, revealed the influence of environment. Best values of heritability were noted for 100-seed weight (92.75%) pod width (67.17%), and empty pod weight (53.68%). The height value of heritability noted for pod width, and weight of empty pod reflected the predominance of heritable variation for this trait. High heritability does not necessarily mean high genetic gain. The utility of heritability estimates is, therefore, increased when they are used to estimate genetic advance (Johnson et al., 1955), which indicates the degree of gain in a character obtained under a particular selection pressure.

Low heritability for weight, seed number and infected seed number per pod can be explained by the loss of weight and seed by abortion and hard infestation of seed. Heritability is important in tree breeding as it provides an index of the relative role of heredity and environment in the expression of various traits (Nanson, 1970). Johnson *et al.* (1955) found that heritability value along with estimates of genotypic gain were more useful than heritability alone in predicting the effect of selection. High heritability estimates associated with high genotypic conventional gain were obtained for the traits: 100-seed weight (92.75 and 17.20%), empty pod weight (53.68 and 21.18%) and pod weight (46.45 and 16.13%).

Genotypic and phenotypic correlation coefficients (Table 6) between traits revealed a positive and high correlation with all traits, except for infected seed number. Correlation coefficient at phenotypic level was higher than correlation coefficients at genotypic level for

Table 6: Genotypic (G) and phenotypic (P) correlation of coefficients among pod traits of Acacia tortilis (Forsk.) Hayne ssp. raddiana (Savi) Brenan. α=0.001

Traits	Type	LP (cm)	WP (mm)	WtP (g)	WtS/P (g)	WtEpP (g)	SN/P	InSN/P
LP (cm)	G	0.191***	0.605***	0.505***	0.567***	0.578***	0.247***	0.247***
` ′	P	0.178***	0.713***	0.596***	0.675***	0.676***	0.325***	0.174***
WP (mm)	G		0.640***	0.525***	0.606***	0.121***	-0.039	0.543***
. ,	P		0.488***	0.397***	0.469***	0.078***	-0.011	0.445***
WtP (g)	G			0.776***	0.879***	0.583***	0.129***	0.551***
	P			0.835***	0.901***	0.678***	0.218***	0.384***
WtS/P(g)	G				0.415***	0.243***	0.072***	0.940***
	P				0.597***	0.697***	0.108***	0.464***
WtEpP (g)	G					0.280***	0.251***	0.942***
	P					0.553***	0.258***	0.267***
SN/P	G						0.177***	0.696***
	P						0.403***	0.050
InSN/P	G							-0.156
	P							-0.075

\*\*\*Significant at  $\alpha$ = 0.001. LP: Pod length; WP: Pod width; WtP: Pod weight; WtS/P: Seeds weight per pod WtEpP: Empty pod weight; SN/P: Seed number per pod; InSN/P: Infected seed number per pod Wt100: 100-seed weight

different traits under study excluding pod width, where there was a slight increase of genotypic correlation coefficients. This clearly reflects genotypic influence for pod width and environmental influence for all the remaining traits.

#### CONCLUSION

Genetic diversity analysis based on morpho-metric traits of Acacia raddiana provenances in Morocco provides basic information for tree improvement and conservation programs. This research can help to identify the better genotypes of Acacia raddiana what may be used for further improvement of Acacia. Present study on provenances showed that all morpho-metric characters are influenced by environment (soil quality, temperature and annual rainfall) and genetic factors. We have observed significant variations within provenances in all traits and only in six traits among provenance. The hyper-variability of molecular markers constitutes great advantages for these types of studies. Further research including available ISSR (Inter Simple Sequence Repeats) and microsatellites marker would hopefully provide additional information. Possibility of Acacia senegal (L.) Willd SSR (Assoumane et al., 2009) transferability to Acacia raddiana constitutes a strategic tool for this species for which SSRs have not been developed yet. On the other hand, given this possibility, an additional value well bee obtaining because of the importance of having common markers between species to be used as bridge markers in genetic mapping (Azpilicueta et al., 2004).

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