

*Phenotypic biodiversity of an endemic wild pear, *Pyrus mamorensis* Trab., in North-Western Morocco using morphological descriptors*

Asma Ait Said, Ahmed Oukabli, Fatima Gaboun, Marie H el ene Simard & Cherkaoui El Modafar

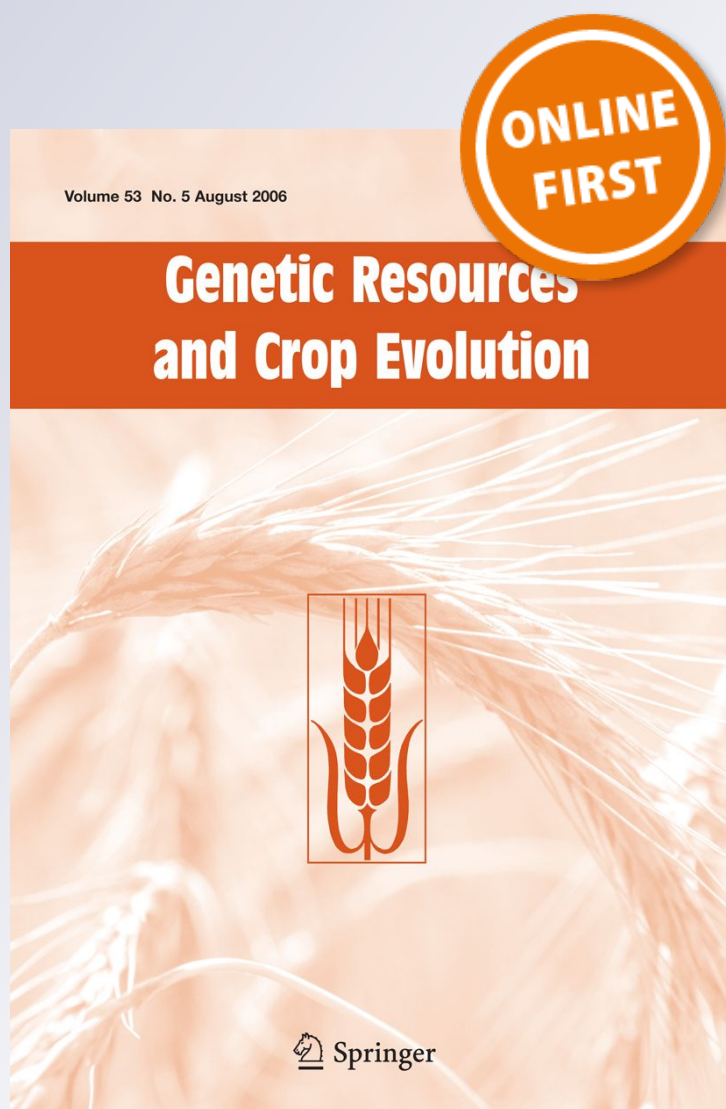
Genetic Resources and Crop Evolution

An International Journal

ISSN 0925-9864

Genet Resour Crop Evol

DOI 10.1007/s10722-012-9889-4



Your article is protected by copyright and all rights are held exclusively by Springer Science +Business Media Dordrecht. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your work, please use the accepted author's version for posting to your own website or your institution's repository. You may further deposit the accepted author's version on a funder's repository at a funder's request, provided it is not made publicly available until 12 months after publication.

Phenotypic biodiversity of an endemic wild pear, *Pyrus mamorensis* Trab., in North-Western Morocco using morphological descriptors

Asma Ait Said · Ahmed Oukabli ·
Fatima Gaboun · Marie H el ene Simard ·
Cherkaoui El Modafar

Received: 16 April 2012 / Accepted: 23 July 2012
  Springer Science+Business Media Dordrecht 2012

Abstract In this paper, we report on morphological diversity, ecology and flowering phenological records of an endangered and an endemic species of Morocco *Pyrus mamorensis* Trab. For this study, a survey was undertaken in North-Western Morocco (Mamora forest and Za er region) whereby 67 accessions of *P. mamorensis* were sampled. A set of 33 genotypes of *Pyrus* spp. from the international collection INRA-Angers (France) of the genus *Pyrus* were also analyzed. The variability of the total of 100 accessions was assessed by twenty-six characters including fourteen qualitative and twelve quantitative traits.

The results demonstrated high levels of morphological variation in the sampled accessions of *P. mamorensis* and established the relationships among the local germplasm using the UPGMA cluster analysis and the factorial discriminant analysis. Furthermore, we were able to distinguish three types of flowering in the Mamora forest: early, intermediate and late flowering type. Otherwise, the UPGMA classification comparing local accessions of *P. mamorensis* to the international collection led to the formation of four major groups showing the closest correspondence to their geographical origins: (1) The North African species represented by *P. mamorensis*, (2) The East Asian pear species, (3) The West Asian species and (4) The European species. These findings suggest the importance of implementing a conservation strategy preserving the genetic resources of wild pear in North-Western Morocco.

A. Ait Said · C. El Modafar
Laboratoire de Biotechnologie Valorisation et Protection
des Agroressources, Facult  des Sciences et Techniques
Gu eliz, Universit  Cadi Ayyad, B.P. 549, 40 000
Marrakech, Morocco

A. Ait Said · A. Oukabli ( )
UR Am lioration des Plantes et Conservation des
Ressources Phytog n tiques, INRA, B.P. 578,
Mekn s, Morocco
e-mail: oukabli2001@yahoo.fr

A. Ait Said · M. H. Simard
UMR GenHort INRA-INH-UA, Unit  d'Am lioration des
Esp ces Fruiti res et Ornementales, 42 Rue G. Morel,
B.P. 60057, 49071 Beaucoz  Cedex, France

F. Gaboun
UR Biotechnologie, CRRA, INRA, B.P. 415,
Rabat, Morocco

Keywords Factorial discriminant analysis ·
Genetic conservation · Morphological diversity ·
Phenological records · *Pyrus mamorensis* Trab. ·
UPGMA classification

Introduction

The genus *Pyrus* belongs to the subfamily Maloideae in the family Rosaceae. The origin of the genus is considered to be in the mountainous area of western

China and is known to evolve and spread eastward and westward (Challice and Westwood 1973; Hummer and Postman 2003). In spite of the wide geographic distribution of *Pyrus*, species are intercrossable and there are no major incompatibility barriers to interspecific hybridization (Westwood and Bjornstad 1971). Furthermore, the narrow morphological diversity and the lack of distinguishing characters among species have been reported (Westwood 1982; Voltas et al. 2007). Therefore, classification of pear species is very problematic and is often confusing and giving similar taxa designated as different species by some authors (Aldasoro et al. 1996). Twenty-two main species have been reviewed and listed by Challice and Westwood (1973) with their geographical origins. It was reported also that *Pyrus* has natural occurrences in the wild state in Europe, temperate Asia and mountainous areas of northern Africa.

Among these species, *Pyrus mamorensis* Trabut (1916), a wild pear endemic to Morocco has been mentioned. Commonly called 'Njache', this tree grows spontaneously in the plains and low mountains. Fennane et al. (1999) classified *P. mamorensis* as a rare wild tree. This later was reported by Fennane and Ibn Tattou (1998) in the North-Western Morocco and especially in the Cork-oak forests such as the Mamora forest. It's the only spontaneous arborescent species associated with *Quercus suber* L., in scattered or grouped trees (Metro and Sauvage 1955). However, these ecosystems seem to undergo a very strong regression due to the combined effect of overgrazing, drought, and reforestation (Bakry 1996; Belghazi et al. 2008). Under these conditions, the potential of natural regeneration of wild species is decreasing rapidly.

Until now, no investigation on the morphological characterization of *P. mamorensis* in Morocco was made. Therefore, it is necessary to assess the wild genetic diversity of this local species in order to establish an *ex situ* collection and to prevent its genetic erosion.

The main objectives of the present study were (1) to assess the genetic diversity present at the morphological level within and among two populations of wild *P. mamorensis* in North-Western Morocco; (2) to compare the local accessions of *P. mamorensis* to the international collection of *Pyrus* maintained in the research station INRA-Angers (France) and (3) to investigate the usefulness of morphological characters in the classification of *Pyrus* species.

Materials and methods

Analyzed populations and sampling

Field expeditions were started in 2008 with the aim of collecting *P. mamorensis* accessions throughout North-Western Morocco. Sites were selected on the base of indigenous information and previous literature (Fennane and Ibn Tattou 1998; Fennane et al. 1999). Collections were made from wild habitats which were concentrated in two principal regions: The Mamora forest and Zaër region. A total of 67 accessions of *P. mamorensis* (consisting each of an individual tree) collected according to population's size were sampled: 56 from the Mamora forest and 11 from Zaër region. Trees were chosen randomly, with no phenotypical selection, and were at least 50 m away from each other to avoid sampling from related individuals. Within the fieldwork, the accessions were located with a hand-held global positioning system (GPS). Location information of the sampled stands is provided in Table 1.

Furthermore, a set of 33 genotypes of *Pyrus* spp. from the international collection of the genus *Pyrus* held in the research station INRA-Angers were used (Table 2).

Traits were measured and evaluated separately on a representative population of leaf-shoot and fruit. For each accession and for each character studied, we made 15 individual measurements. A sample of 15 adult leaves, 15 one-year-old shoots and 15 mature fruits per tree were evaluated and measured using a caliper. All of them were taken from all tree orientations and middle parts of shoots, and only healthy and undamaged ones were selected.

Morphological descriptors

A total of 26 morphological characters including 14 binary or multistate traits and 12 quantitative variables were analyzed, following both the guidelines of the International Union for the Protection of New Varieties of Plants (UPOV 2000) and the Descriptor List for Pear of the Commission of European Communities and the International Plant Genetic Resources Institute (CEC and IPGRI 1983). Some relevant traits for the identification of wild forms, such as presence of thorns on young shoots or leaf hairiness, were also added (Wagner 1995). The analysis included three characters

Table 1 Informations on *P. mamorensis* stand's location in North-Western Morocco

Species	Stand	Number of accessions	Region	Latitude (°N)	Longitude (°W)
<i>Pyrus mamorensis</i>	Ait Malek	8	Mamora forest, canton D	33.58	6.25
	Sidi Bou azza (Oued Tiflet)	2	Mamora forest, canton D	34.03	6.21
	Ain Johra (Hefrat N'Jach)	11	Mamora forest, canton C	34.06	6.21
	Ch'abat Awid al Ma	11	Mamora forest, canton C	34.04	6.18
	Sidi Qaddour	6	Mamora forest, canton C	34.06	6.23
	Sidi Allal Bahraoui (Sale's route)	10	Mamora forest, canton B	34.02	6.33
	Dayet Al Haila (Kenitra's route)	8	Mamora forest, canton A	34.09	6.35
	Had Laghoualem	11	Zaër	33.22	6.41

Table 2 List of *Pyrus* spp. from the international collection INRA-Angers (France) used as reference samples in this study

Species	Acronym	Number of accessions	Genotype code
<i>Pyrus communis</i> L.	PC	5	P10-1; P11-2; P2395; P2420; P5-1
<i>Pyrus betulaefolia</i> Bge.	PB	5	P1587; P2419; P252; P2631; P3502
<i>Pyrus calleryana</i> Decne.	PCal	5	P1541; P2418; P2445; P3311; P3313
<i>Pyrus syriaca</i> Boiss.	PS	4	P2400; P2403; P2635; P3515
<i>Pyrus nivalis</i> Jacq.	PN	4	P1967; P1990; P2470; P2472
<i>Pyrus persica</i> Pers.	PaP	2	P251; P264
<i>Pyrus amygdaliformis</i> Vill.	PA	1	P257
<i>Pyrus salicifolia</i> Pall.	PSal	1	P1851
<i>Pyrus elaeagnifolia</i> Pall.	PE	1	P1588
<i>Pyrus cordata</i> Desv.	PcC	1	P3511
<i>Pyrus longipes</i> Coss. et Dur.	PL	1	P1855
<i>Pyrus pashia</i> Ham. ex D. Don	PP	1	P707
<i>Pyrus malifolia</i> Spach	PM	1	P463
<i>Pyrus gharbiana</i> Trab.	PG	1	P1709

of the tree, thirteen of the leaf and the shoot, and ten of the fruit. The full lists are reported in Tables 3 and 4.

Flowering periods of *P. mamorensis* in the Mamora forest were also recorded along three years (2008–2010).

Here we give an overview of flowering phenological records of *P. mamorensis* which cover the first flower, full bloom, and the end of flowering. Whilst the first flowering dates were reported as the first flower seen, the full bloom pertain to the date when 50 % of the flowers were fully opened, and the end of flowering was related to the date when at least 95 % of the flowers were withered.

Thus, the 'flowering spread' or 'flowering season' was recorded as the number of days between the first and last flowering of individuals in a population (Elzinga et al. 2007). The flowering season was

recorded because it is seen as a better indicator of chill than a single flowering date (Sunley et al. 2006). This study was carried out *in situ* on selected and labeled trees.

Statistical analysis

Variations among accessions for each morphological trait were analyzed using the analysis of variance (ANOVA). Coefficient of variation (CV) was computed, and means were compared using *t* tests for the two populations of *P. mamorensis*. Similarly, the relevance of categorical variables was established with the Chi-squared values which tested the independence between each categorical variable and the wild stands surveyed.

Table 3 List of tree, leaf–shoot and fruit quantitative traits used as descriptors

Characters	Acronym
Leaf–shoot quantitative traits	
Internodes length (mm)	InL
Leaf blade length (mm)	LL
Leaf blade width (mm)	LW
Ratio width/length	LW/LL
Petiole length (mm)	PL
Fruit quantitative traits	
Fruit length (mm)	FL
Fruit diameter (mm)	FD
Ratio diameter/length	FD/FL
Peduncle length (mm)	PdL
Peduncle diameter (mm)	PdD
Ratio diameter/length of peduncle	PdD/PdL
Ratio peduncle length/fruit length	PdL/FL

In order to find the main variation trends between fruit and leaf continuous characters and to evaluate their correlation, data were processed according to factorial discriminant analysis (FDA), scores and factor loadings were calculated after standardization. The FDA was performed on the matrix of average values of quantitative traits. Canonical discriminant analysis was used to perform graphical representation

of the four cantons of Mamora forest on a two-dimensional graph.

The data was assigned to groups or categories after standardization in order to compute similarity between accessions using the Simple Matching Coefficient (Sneath and Sokal 1973). The accessions were then clustered by the Unweighted Pair-Group Method with Arithmetic Averages (UPGMA). A cophenetic value matrix (Sneath and Sokal 1973) of the UPGMA clustering was used to test for the goodness-of-fit of the clustering to the similarity matrix on which it was based, by computing the product-moment correlation (r) with 1,000 permutations (Mantel 1967).

All analyses were carried out using the SAS v.9.1 (SAS 2004), GenStat v.10.2 (Genstat 2007), and the NTSYSpc v.2.02i (NTSYSpc NTSYS 1998) packages.

Results and discussion

Ecology and phenological observations of *P. mamorensis* in North-Western Morocco

Ecology of P. mamorensis in North-Western Morocco

The study area in North-Western Morocco extends between 6°18' and 6°41' West and between 33°22' and 34°09' North. The main ecological characteristics of

Table 4 List of tree, leaf–shoot and fruit qualitative traits with character states used as descriptors

Character	Character states	Acronym
Tree vigor	(1) Weak (2) medium (3) strong (4) very strong	TV
Tree branching	(1) Very weak (2) weak (3) medium (4) strong (5) very strong	TB
Tree habit	(1) Fastigiate (2) upright (3) semi-upright (4) spreading (5) drooping (6) weeping	TH
Young shoot: predominant color on sunny side	(1) Grey green (2) grey brown (3) medium brown (4) orange brown (5) brown red (6) brown purple (7) dark brown	YSC
Young shoot: presence of thorns	(1) Absent (2) present	YST
Leaf blade: shape of base	(1) Acute (2) right-angled (3) obtuse (4) truncate (5) cordate	LBB
Leaf blade: shape of apex	(1) Acute (2) right-angled (3) obtuse (4) rounded	LBA
Leaf blade: incisions of margin	(1) Absent (2) crenate (3) bluntly serrate (4) sharply serrate	LBM
Leaf color	(1) Pale green (2) light green (3) medium green (4) dark green (5) glaucous	LC
Leaf blade: presence of hairs	(1) Absent (2) present	LBH
Petiole: presence of stipules	(1) Absent (2) present	PS
Fruit: position of maximum diameter	(1) In middle (2) slightly towards calyx (3) clearly towards calyx	FMD
Fruit: peel color	(1) Light brown (2) brown (3) brown-green (4) orange brown (5) yellow (6) green yellow (7) orange (8) green (9) light green	FPC
Fruit calyx	(1) Deciduous or semi-persistent (2) persistent	FC

Table 5 Ecological characteristics of Mamora forest and Zaër region, the surveyed stands in North-Western Morocco

Stand	Area (ha)	Elevation (m)	Range of bioclimate ^a	Soil type	Native tree species	Introduced tree species
Mamora forest	134.000	116–231	Temperate sub-humid (cantons A and B) Semi-arid (cantons C and D)	Complex of red sand and clays covered by a light brown sands ^b	Main species: <i>Quercus suber</i> L. Associated species: <i>Pyrus mamorensis</i> Trab. <i>Olea europaea</i> L. subsp. <i>oleaster</i> (Hoff. et Link) Greuter et Burdet	Eucalyptus Acacia
Zaër region	108.955	512	Semi-arid	Red clays covered by leached sand	Main species: <i>Quercus suber</i> L. <i>Quercus ilex</i> L. <i>Tetraclinis articulata</i> (Vahl) Mast. Associated species: <i>Pyrus mamorensis</i> Trab. <i>Olea europaea</i> L. var. <i>sylvestris</i> (Mill.) Brot.	NR

NR not recorded

^a Benabid and Fennane (1994)

^b Textier et al. (1992)

Mamora forest and Zaër region are listed in Table 5. Over the last decades, these surveyed ecosystems have undergone a very strong regression and have become fragmented into relatively small and isolated patches. The almost total absence of natural regeneration is among several reasons for regression. This regression is due to anthropogenic pressures of many small communities surrounding these forests; such as overgrazing and the expansion of lands used for agriculture and urbanization. Besides, these ecosystems are threatened by extreme consecutive years of drought, and insect infestations. Populations of *P. mamorensis* located in the Mamora forest and Zaër region consisted of scattered or grouped trees. Our survey revealed that wild pear stands in Zaër region have become rare. Only eleven accessions were found, located on marginal or steep lands. Furthermore, some stands, in both Mamora forest and Zaër region, consisted of small trees of wild pear, stunted by overgrazing and consecutive droughts.

Flowering period of *P. mamorensis*

Results of monitoring seasonal flowering of *P. mamorensis* population located in the Mamora forest

are presented in Fig. 1. Three types of flowering periods appear: early, intermediate and late flowering. The starting dates for flowering of *P. mamorensis*, along the 3 years, were estimated to be 10–17 January for the early-flowering type (as an extreme earliness in flowering), 23 January–6 February for the intermediate-flowering type, and 8–18 February for the late-flowering type (as an extreme lateness in flowering). It is assumed, that the changes in first flowering dates describe the phenological behavior of an entire population. Nevertheless, first flowering dates may be susceptible to undesirable confounding effects. Thus, when interpreting changes in first flowering dates we should consider the entire flowering season to avoid the confounding effects of population size and sampling frequency (Sunley et al. 2006).

Our results show that some accessions of *P. mamorensis* could flower over only a very short duration (7 days recorded for the late-flowering type in 2010), whereas others flower over a much longer period of the flowering season (31 days recorded for the early-flowering type in 2008). Flowering times vary from year to year in the Mamora forest depending on the climatic conditions of the year. The first flowering dates in 2010 occurred earlier than they did

Type	January	February	March
10.....20.....30.10.....20.....2910.....20.....30.
Early-flowering type			
Intermediate-flowering type			
Late-flowering type			

✦ Full bloom

Fig. 1 Flowering dates of *P. mamorensis* in the Mamora forest. Dates are the average of three years (2008, 2009 and 2010)

in 2008 and 2009. The same responses were observed for the flowering seasons, short durations were recorded for the 3 flowering types in 2009 compared with 2008. The earliest flowering of *P. mamorensis* translates the low chilling requirement of this species. In woody plants, air temperature during the winter, is probably the most important environmental factor determining the start of plant development in a flowering season. However, development might be modified by other climatic factors. For instance, air humidity, soil temperature and other soil characteristics (Wielgolaski 1999).

Morphological analysis of *P. mamorensis*

High levels of morphological variation in the sampled accessions were found. The average values for all leaf-shoot and fruit morphological characteristics observed were statistically different at $P \leq 0.001$ (Table 6). Peduncle shape (PdD/PdL) had the highest variation of all (51.19 %). The quotient (PdL/FL) and the internodes length also presented a high variability. The lowest coefficient of variation was observed for fruit shape and width.

According to Krause et al. (2007), a high morphological diversity was observed within the *Pyrus pashia* trees in central Bhutan. Other tree species have also shown high morphological variation in populations natives to the Mediterranean area; for instance, cork-oak (Sbay et al. 1996), Aleppo pine (Boulli et al. 2001) and carob (Naghmouchi et al. 2009). However, the Chi-squared values revealed that most categorical traits were useless in differentiating wild stands except tree vigor (TV) and its branching (TB) (not shown).

The comparison of the means among populations revealed significant difference on most traits ($P < 0.05$), except the petiole length and peduncle length, diameter and shape which were not

significantly different among population (Table 6). Variation in climatic conditions (rainfall, temperatures...) might explain the differences in morphological traits between populations. In contrast, accessions from the Mamora forest trees had longer internodes, smaller fruits and larger leaves than their counterparts from Zaër region.

Climate conditions appear to be important determinants of the morphological traits of Mediterranean trees. It has been shown in early studies (in almond and Aleppo pine species) that variation in phenotypic traits is frequently determined by environmental gradients such as rainfall (Lansari et al. 1994; Talhouk et al. 2000) and altitude (Boulli et al. 2001).

Over all analyzed accessions, *P. mamorensis* had small fruits, the average of FL and FD were 20.1 and 17.6 mm respectively. According to Wagner (1995, 1996) and Hofmann (1993), these sizes are typical for wild pear fruits. In addition, the fruits were mostly pear-shaped (60.3 %), less frequent, they were apple-shaped (39.7 %); average value of the fruit shape FD/FL was 0.9 mm with range 0.7–1.13; and the position of maximum diameter was slightly towards calyx in 78.6 % of analyzed fruits. Peduncle lengths (PdL) were often short or intermediate (91.0 %) with an intermediate width, and exceptionally long peduncles (8.9 %).

In studied populations, leaves were mostly ovate (74.6 %) and orbicular 25.4 %. The leaf blades were 30.41–50.27 mm long and 20.43–33.27 mm wide. Further, a vast majority (75 %) had short-to-intermediate petiole lengths (PL). *P. mamorensis* leaf base shapes were different within population ranging from right-angled, obtuse, truncate to acute but mostly obtuse (46.43 %); while leaf margins were crenate (35.71 %) or bluntly serrate (64.29 %). Moreover, leaf apices were represented by 3 different shapes: acute, right-angled and bluntly serrate. The distribution of serrate leaf apices was dominant (80.4 %).

Table 6 Means, standard deviations, coefficient of variation and *F* values from one-way ANOVA of 12 morphological characters determined for leaf-shoot and fruit characters observed in 67 accessions of *P. mamorensis*

Region	Leaf-shoot quantitative traits						Fruit quantitative traits					
	InL	LL	LW	LW/LL	PL	FL	FD	FD/FL	PdL	PdD	PdD/PdL	PdL/FL
Mamora forest	21.51 ± 0.331a	41.29 ± 0.219a	31.06 ± 0.175a	0.76 ± 0.004a	40.65 ± 0.004a	19.8 ± 0.116a	17.03 ± 0.082a	0.87 ± 0.003a	24.59 ± 0.248a	2.12 ± 0.022a	0.09 ± 0.002a	1.27 ± 0.014a
Zaïer	18.59 ± 0.507b	40.32 ± 0.529b	27.09 ± 0.402b	0.7 ± 0.03b	41.38 ± 0.765a	21.4 ± 0.272b	20.38 ± 0.213b	0.96 ± 0.008b	25.36 ± 0.6a	2.05 ± 0.02a	0.09 ± 0.003a	1.22 ± 0.036b
CV	43.33	15.57	17.4	26.81	30.22	16.87	15.39	12.65	28.55	27.99	51.19	32.56
<i>F</i> value	6.09***	10.52***	12.07***	2.97***	9.61***	34.34***	20.92***	25.2***	22.73***	3.96***	8.42***	27.46***

Differences between regions of accession are indicated by *t* test at *P* < 0.05

*** Statistically significant differences between accessions at *P* ≤ 0.001

All of the studied accessions of *P. mamorensis* had thorns on their shoots. The presence of thorns, however, cannot be interpreted as an unambiguous indicator of wildness, because they are also present in naturalized edible varieties (Wagner 1995). Other characteristics have negligible discriminatory power, according to the Chi-squared values for the categorical traits and the *F* values for the continuous traits.

Otherwise, the structure of the Mamora forest population was carried out and the relationships among *P. mamorensis* accessions were established. As a general multivariate ordination method, the factorial discriminant analysis was applied to the surveyed population in the Mamora forest based on continuous morphological traits.

The results revealed that both fruit and leaf characters were prevalent in the first and the second discriminant functions, and contributed most to the total variation. Whereas some variables were significantly correlated, none have an *r*² higher than 0.63 (Table 7).

The two canonical discriminant functions explained 90 % of the total variation contributed by all quantitative traits. Fruit diameter and peduncle diameter have the highest loading in the first discriminant function that accounted for a variation of 49 %. The second discriminant function contributing 41 %, included leaf length and leaf blade shape (Table 7).

Table 7 Correlation coefficients between the quantitative characters and the 2 factorial axes in the 7 stands in Mamora forest

Variables	Discriminant functions	
	[1]	[2]
FD	0.5412	0.1897
PdD	0.6181	0.0570
FD/FL	-0.1159	-0.2730
InL	-0.0762	0.2184
FL	0.4966	0.3607
LL	0.2282	0.6322
PL	0.2486	0.2871
PdL	-0.0449	-0.0775
LW	0.1837	0.1778
LW/LL	0.2458	-0.5709
Cumulative total variance (%) ^a	49	41

Coefficients >0.5 are in bold

^a The first and the second discriminant functions contributed to 49 and 41 % respectively

The plot of the two canonical discriminant functions (Fig. 2) showed a dispersion of accessions in correlation with their geographical location in the Mamora forest (canton A, B, C and D).

Three groups could be differentiated; the first grouped all the accessions from the canton A and B, they were negatively correlated to the second discriminant function. Trees from the canton C and D were considered as rather singular.

We then used the UPGMA cluster analysis derived from morphological data, assigned to categories after standardization, in order to identify accessions with the highest level of similarity and evaluate their phenotypic diversity (Fig. 3). The evaluated Genetic distances were calculated using both qualitative and quantitative traits, and the average distance is (0.422 ± 0.545) . Moreover, the cophenetic correlation between the dendrogram and the similarity matrix revealed a good degree of fit ($r = 0.536$; $P < 0.002$).

The morphological phenogram shows the minimum distance between clusters and the extent of morphological relationship between accessions within the Mamora forest. All the accessions used in the current study have between 35 and 79 % genetic

similarity. At 39 % of similarity, the resulting dendrogram allowed to distinguish three groups for *P. mamorensis* in the Mamora forest whereas five accessions (Ma36, Ma02, Ma09, Ma35 and Ma52) remained ungrouped. A noteworthy fact is that accessions represented in the same morphological group were all from stands and cantons. Hence, for all 20 traits together, there were no consistent differences between cantons. The highest proportion of similarity was observed between the two accessions Ma15 and Ma55 with 79 % of similarity. The next four most similar pairs had 73.5 % similarity (Ma07/Ma43, Ma03/Ma29, Ma47/Ma48, and Ma17/Ma23). Interestingly, the accessions that had the highest similarity were from different stand and/or canton, except for the pair Ma47/Ma48 which was sampled from the same stand.

Evaluation of phenotypic diversity within the 56 accessions of *P. mamorensis* in the Mamora forest assumes that variations in morphological properties existed among accessions with respect to the 20 traits that were evaluated.

Assigning local accessions of *P. mamorensis* by comparing to the international collection of *Pyrus* (INRA-Angers, France)

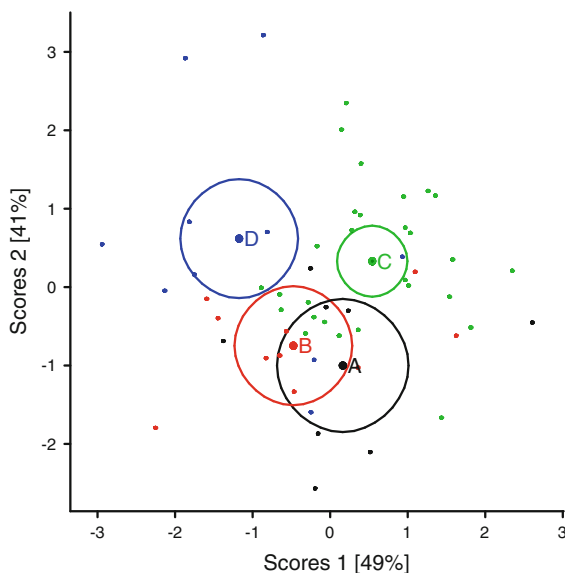


Fig. 2 Projection of the Mamora forest population onto the plane defined by the first two canonical discriminant functions (Scores 1 and 2) performed on the basis of the leaf shoot and fruit quantitative traits. **A** canton A, **B** canton B, **C** canton C, **D** canton D

UPGMA cluster analysis of 89 *Pyrus* spp. accessions including 56 local samples of *P. mamorensis* and 33 reference samples from INRA-Angers collection was also performed in this study (Fig. 4). Data regarding the 11 accessions of *P. mamorensis* sampled from Zaër region were excluded for insufficient fruit data.

The calculation of genetic distance based upon 23 characters (continuous and multistate) was carried out after standardizing, and the average distance was (0.385 ± 159.52) . The cophenetic correlation between the dendrogram and the similarity matrix revealed a good degree of fit ($r = 0.9182$; $P < 0.002$). The resulting classification shows the closest correspondence to the geographical origins of the species of *Pyrus* as reviewed by Challice and Westwood (1973). In fact, the dendrogram obtained from our data-set shows four principal groups of species: (1) the North African species (2) the East Asian pea species (3) the West Asian species and (4) the European species.

The first group represented only by *P. mamorensis*, as North African species, reveals close relationships between accessions from the Mamora forest.

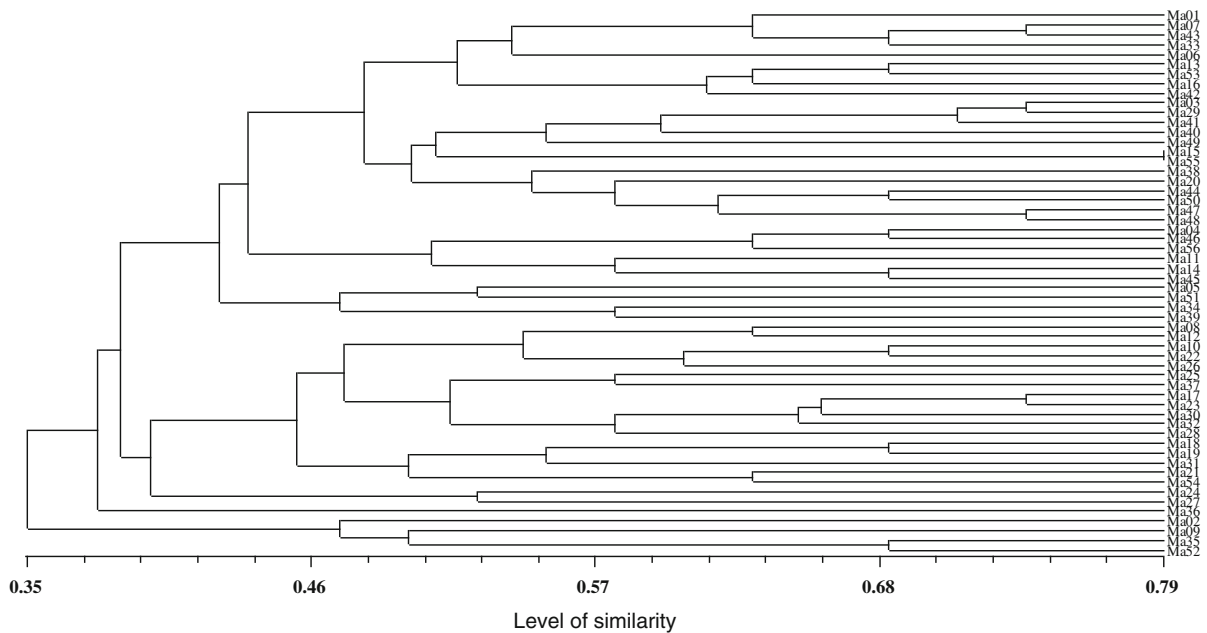


Fig. 3 UPGMA cluster analysis of *P. mamorensis* (Mamora population) based on genetic distances evaluated using both qualitative and quantitative morphological data after

standardization. Distances were calculated using the Simple Matching Coefficient (Rohlf 1998)

All samples of *P. mamorensis* together had a similarity of 43 % and form a fairly distinguished cluster. Several pairs of these accessions have the highest proportion of similarity with 86 %. Besides, at 43 % of similarity, *P. mamorensis* seems to tie quite closely to an European species *Pyrus cordata* rather than the North African species remained (*Pyrus gharbiana* and *Pyrus longipes*). This result is, indeed, supported by Metro and Sauvage (1955) who described *P. mamorensis* as a close neighbor species to *Pyrus cordata*.

In addition, Challice and Westwood (1973) reported that *P. cordata* is related to East North African species and was interpreted as a probable central position of this species in the evolution of species within *Pyrus*.

The East Asian pea species group includes *Pyrus calleryana* and *Pyrus betulaefolia* clustered into phenotypic close pairs. For instance, genotypes of *Pyrus betulaefolia* P1587/P2631 and *Pyrus calleryana* P3313/P2418 were observed to be morphologically close in the dendrogram.

The West Asian species cluster contains accessions of *Pyrus syriaca*, *Pyrus elaeagnifolia*, *Pyrus salicifolia* and *Pyrus amygdaliformis*. The highest proportion

of similarity reported in this group was with 81 % between the two genotypes of *Pyrus syriaca* P3515 and P2403.

The European species form a heterogenic cluster with *Pyrus communis* having greater affinity with *Pyrus nivalis* (64 % of similarity between P2420 and P1990). However, the European group contains seriously misplaced species. Some genotypes cut across their geographical distributions such as P707 and P1855 which belong, respectively, to a medium to large-fruited East Asian and North African species.

Interestingly, it was also observed that genotypes (P1709 and P463) that stood out in the dendrogram and yielded 38 % of similarity were from different species.

Hence, although some relationships were confirmed by this data-set, it would be fair to say that several discrepancies were also present. These observed inconsistencies between some genotypes of *Pyrus* species assume that the taxonomy of the genus *Pyrus* is complex. A considerable number of intermediate forms and more or less stable segregants has arisen causing obvious taxonomic difficulties (Browicz 1993).

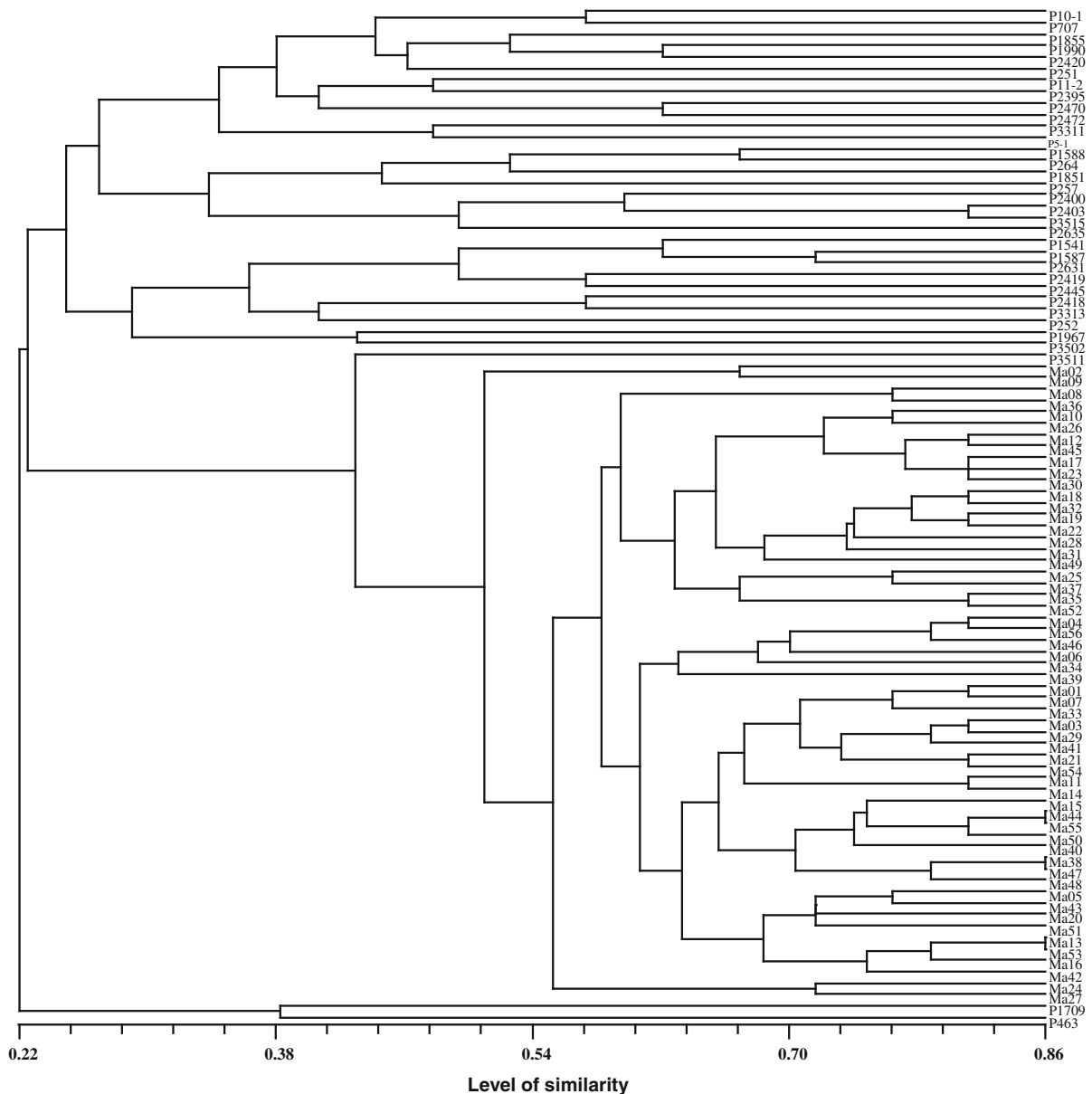


Fig. 4 UPGMA cluster analysis of 100 *Pyrus* spp. accessions (local and reference collection) used in this study. Genetic distances were evaluated using both qualitative and quantitative

morphological data after standardization. Distances were obtained using the Simple Matching Coefficient (Rohlf 1998)

Apart from the fact that the morphological dendrogram generated was able to re-order accessions into distinct clusters and provided an overall pattern of variation as well as the degree of relatedness among accessions, the current study has shown that the use of morphological grouping could not provide convincing discriminatory evidence in the classification of pear accessions.

In fact, it would be unwise to assume that a useful classification will result from the use of available phenotypic traits. The use of morphometric characters combined to agronomic traits and molecular markers might give rise to a different hierarchical pattern which could have higher information content and might not be prone to large environmental variation.

Conclusion

The surveyed ecosystems in North-Western Morocco undergo a very strong regression due mainly to anthropogenic pressures of many small communities surrounding these natural stands. Our investigation on the morphological characterization of *P. mamorensis* demonstrated a large diversity and allowed us to assess the phenotypic biodiversity of this local species. These findings suggest the importance of preserving the genetic resources of wild pear in this area in order to establish an *ex situ* collection; and could be a starting point for further studies, using for instance molecular markers with the aim of implementing a conservation strategy that prevents genetic erosion, and consequently the reduction of the biodiversity of this species in North-Western Morocco.

Acknowledgments This research was supported by PRAD N°190-89 TC project involving the UMR GenHort from Institut National de Recherche Agronomique Angers, France and the UR: APCRG from Institut National de Recherche Agronomique Meknes, Morocco.

References

- Aldasoro JJ, Aedo C, Muñoz Garmendia F (1996) The genus *Pyrus* L. (Rosaceae) in south-west Europe and North Africa. *Bot J Linn Soc* 121:143–158
- Bakry M (1996) Dépérissement du chêne liège (*Quercus suber*): symptômes. Thèse de 3ème cycle, Institut Agronomique Vétérinaire Hassan II, Rabat
- Belghazi B, Ezzahiri M, Ouassou A, Achbah M, El Yousfi SM, Belghazi T (2008) Comportement de quelques clones d'Eucalyptus dans la forêt de la Mâamora (Maroc). *Cahiers Agric*, vol 17, n° 1
- Benabid A, Fennane M (1994) Connaissances sur la végétation du Maroc: phytogéographie, phytosociologie et séries de végétation. *Lazaroa* 14:21–97
- Boulli A, Baaziz M, M'hirit O (2001) Polymorphism of natural populations of *Pinus halepensis* Mill. in Morocco as revealed by morphological characters. *Euphytica* 119: 309–316
- Browicz K (1993) Conspect and chorology of the genus *Pyrus* L. *Arboretum Kórnickie* 38:17–33
- CEC and IPGRI (1983) Descriptor list for pear (*Pyrus*). International Board for Plant Genetic Resources, Rome
- Challice JS, Westwood MN (1973) Numerical taxonomic studies of the genus *Pyrus* using both chemical and botanical characters. *Bot J Linn Soc* 67(2):121–148
- Elzinga JA, Atlan A, Biere A, Gigord L, Weis AE, Bernasconi G (2007) Time after time: flowering phenology and biotic interactions. *Trend Eco Evol* 22:432–439
- Fennane M and Ibn Tattou M (1998) Catalogue des Plantes Vasculaires Rares, Menacées ou Endémiques du Maroc. Herbarium Mediterraneum Panormitanum 'Boccone', Palermo, Italy
- Fennane M, Ibn Tattou M, Mathez J, Ouyahya A, El Oualidi J (1999) Flore pratique du Maroc. Manuel de détermination des plantes vasculaires. Institut scientifique
- GenStat (2007) Statistical analysis computer software, trial version 10.2. Lawes Agricultural Trust (Rothamsted Experiment Station), Harpenden Herts, UK
- Hofmann H (1993) Zur Verbreitung und Ökologie der Wildbirne (*Pyrus communis* L.) in Süd-Niedersachsen und Nordhessen sowie ihrer Abgrenzung von verwilderten Kulturbirnen (*Pyrus domestica* Med.). *Mitteilungen der Deutschen Dendrologischen Gesellschaft* 81:27–69
- Hummer KE, Postman JD (2003) *Pyrus* L. Pear. National Clonal Germplasm Repository, Corvallis, Oregon
- Krause S, Hammer K, Buerkert A (2007) Morphological biodiversity and local use of the Himalayan pear (*Pyrus pashia*) in Central Bhutan. *Genet Resour Crop Evol* 54:1245–1254
- Lansari A, Lezzoni AF, Kester DE (1994) Morphological variation within collections of Moroccan almond clones and Mediterranean and North American Cultivars. *Euphytica* 78:27–41
- Mantel NA (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res* 27(2): 209–220
- Metro A, Sauvage C (1955) Flore des végétaux ligneux de la Mamora. Imprimerie Marcel Bon Vesoul, Casablanca
- Naghmouchi S, Khouja ML, Romero A, Tous J, Boussaid M (2009) Tunisian carob (*Ceratonia siliqua* L.) populations: morphological variability of pods and kernel. *Sci Hortic* 121:125–130
- NTSYS (1998) Numerical taxonomic system multivariate data analysis software version 2.02i. Exeter software, New York, USA
- Rohlf F (1998) NTSYSpc: numerical taxonomy and multivariate analysis system. Version 2.02i. Applied Biostatistics Inc., New York
- SAS (2004) SAS Institute Inc. SAS 9.1, Cary, NC, USA
- Sbay H, Ouassou A, Zitane L (1996) Étude du polymorphisme morphologique du chêne liège, Séminaire sur la régénération du chêne liège dans le bassin méditerranéen, Tabarka, 6–9 Nov, Tunisie
- Sneath PHA, Sokal RR (1973) Numerical taxonomy. Freeman, San Francisco
- Sunley RJ, Atkinson CJ, Jones HG (2006) Chill unit models and recent change in the occurrence of winter chill and spring frost in the United Kingdom. *J Hortic Sci Biotechnol* 81:949–958
- Talhok SN, Lubani RT, Baalbaki R, Zurayk R, AlKhatib A, Parmaksizian L, Jaradat AA (2000) Phenotypic diversity and morphological characterization of *Amygdalus* L. species in Lebanon. *Genet Resour Crop Evol* 47:93–104
- Textier JP, Lefevre D, Raynal JP (1992) La formation de la Mamora, le point sur la question du Moulouyen et du Salétien du Maroc Nord-Occidental. *Quaternaire* 3(2): 63–73
- Trabut L (1916) Les poiriers indigènes dans l'Afrique du Nord. *Bull Stat Rech Forest Nord Afrique* 1:115–120
- UPOV (2000) International union for the protection of new varieties of plants: guidelines for the conduct of tests for distinctness, uniformity and stability, Pear (*Pyrus communis* L.), Geneva

- Voltas J, Pemán J, Fusté F (2007) Phenotypic diversity and delimitation between wild and cultivated forms of the genus *Pyrus* in North-eastern Spain based on morphometric analyses. *Genet Resour Crop Evol* 54:1473–1487
- Wagner I (1995) Identifikation von Wildapfel (*Malus sylvestris* (L.) Mill.) und Wildbirne (*Pyrus pyraeaster* (L.) Burgsd.). Voraussetzung zur Generhaltung des einheimischen Wildobstes. *Forstarchiv* 66:39–47
- Wagner I (1996) Zusammenstellung morphologischer Merkmale und ihrer Ausprägungen zur Unterscheidung von Wild- und Kulturformen des Apfel (*Malus*) und des Birnbaumes (*Pyrus*). *Mitteilungen der Deutschen Dendrologischen Gesellschaft* 82:87–108
- Westwood MN (1982) Pear germplasm of the new national clonal repository: its evaluation and use. *Acta Hort* 124:57–65
- Westwood MN, Bjornstad HO (1971) Some fruit characteristics of interspecific hybrids and extent of self-sterility in *Pyrus*. *Bull Torrey Bot Club* 98:22–24
- Wielgolaski FE (1999) Starting dates and basic temperatures in phenological observations of plants. *Int J Biometeorol* 42:158–168