

Genetic diversity of the Algerian peanut population analyzed using morphological markers and seed storage proteins

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Генетическое разнообразие
 алжирской популяции арахиса, изученной
 с использованием морфологических маркеров
 и запасных белков семян

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Background. The peanut is one of the most important oil crops suitable for cultivation in the tropical areas of the world. Despite its agronomic importance, few studies have been carried out to assess the morphogenetic diversity of *Arachis hypogaea* L., especially in East African countries. The major interest of this morphologic study lies in the potential of this species to provide useful genes for the improvement of cultivated peanuts. To date, no study has been performed in Algeria to characterize local peanut varieties.

Materials and methods. Thirty peanut accessions were collected from four principal areas of peanut production in Algeria. Genetic characterization using 15 agronomic characters and 25 morphological descriptors showed a high level of diversity among accessions. Principal Component Analysis and the Hierarchical Ascendant Classification were made to clarify the genetic relationship between peanut accessions.

Results and discussion. Results showed that leaflet size (length and width), seed shape and size, oil content, and branching pattern were the principal characters to discriminate the screened *A. hypogaea* accessions. In addition to that, the weights of 10 pods and 100 seeds were the most variable traits and presented a CV of 42.53% and 40.12%, respectively. On the other hand, total storage proteins extracted were separated using SDS-PAGE and revealed thirty bands that were used to generate a matrix and make a cluster analysis using the UPGMA method, exhibiting different storage proteins compositions. Moreover, the phenotypic diversity observed agrees with the storage protein profile diversity, while the accessions grouped in similar clusters belong to the two subspecies of *A. hypogaea*. The results of the current study show that morphological traits and seed storage proteins can be useful for exploring the diversity among *A. hypogaea* accessions.

Key words: *Arachis hypogaea*, phenotypic diversity, Principal Component Analysis, SDS-PAGE.

Актуальность. Арахис – одна из важнейших масличных культур, пригодных для возделывания в тропических зонах мира. Несмотря на его хозяйственную ценность, были предприняты лишь редкие попытки изучить и оценить морфогенетическое разнообразие *Arachis hypogaea* L., особенно в странах Восточной Африки. Основная цель настоящего морфологического исследования – изучить потенциал данного вида как источника ценных генов для улучшения культурных форм арахиса. До сегодняшнего дня в Алжире не проводились исследования по выявлению характеристик местных сортов арахиса.

Материалы и методы. Тридцать образцов арахиса собрали в четырех основных районах его производства в Алжире. Генетическая характеристика с использованием 15 агрономических и 25 морфологических признаков показала высокий уровень разнообразия среди образцов. Для выяснения генетической взаимосвязи между образцами арахиса провели кластерный анализ и анализ главных компонент.

Результаты и обсуждение. Показано, что размер листочков (длина и ширина), форма и размер семян, содержание масла и характер ветвления были основными признаками, позволяющими различить отобранные образцы *Arachis hypogaea* L. В дополнение к этому, массы 10 бобов и 100 семян были наиболее изменчивыми характеристиками и имели CV 42,53% и 40,12% соответственно. Был проведен анализ запасных белков семян. Все они были разделены с использованием метода SDS-PAGE, который выявил тридцать полос. Последние были использованы для создания матрицы и кластерного анализа методом UPGMA и продемонстрировали различия образцов арахиса по составу запасных белков. Наблюдаемое фенотипическое разнообразие согласуется с разнообразием профиля запасных белков, и группы образцов в сходных кластерах принадлежали двум подвидам *A. hypogaea*. Результаты данного исследования показывают, что морфологические признаки и запасные белки семян могут быть полезны для изучения разнообразия образцов арахиса.

Ключевые слова: *Arachis hypogaea*, фенотипическое разнообразие, анализ главных компонент, SDS-PAGE.

Introduction

Peanut, or groundnut (*Arachis hypogaea* L.), is an important oilseed crop cultivated worldwide. In 2018, 45.9 million tons were produced over an area of 28.5 million hectares (FAOSTAT, 2020). *A. hypogaea* can play an important role in sustainable agricultural development, particularly where drought and salinity frequently limit crop production in the Maghreb and the sub-Saharan regions (Giuffrè et al., 2016). Peanut cultivars have a very narrow genetic base, and there is a lack of information about their morphological and agricultural characteristics. Accurate information on genetic diversity helps the plant breeders in choosing the diverse parents for focused hybridization (Kushwah et al., 2016). Additionally, analysis of storage proteins is considered one of the most powerful methods for describing plant species and lines (Liang et al., 2006). Thus, the aim of the present study was to characterize the peanut accessions currently cultivated in the four main growing areas in Algeria. The specific objectives of the investigation were to estimate phenotypic diversity for different morphological descriptors and agronomic characters, reveal the importance of these traits in addition to their interrelationships, and assess the seed storage protein diversity in peanut for identification of accessions.

Material and methods

Thirty peanut (*A. hypogaea*) accessions were collected from different peanut growing areas in Algeria (Fig. 1), including El Taref, Ghardaïa, El Oued, and Adrar (Table 1). Seedlings were grown and maintained under greenhouse conditions at the National Center for Biotechnology Research in Constantine, Algeria (36°24.75' N, 6°57.05' E, and 773 m above sea level). During their growth, plants were irrigated as needed. Finally, seeds were collected manually, air-dried in the dark, and stored in paper bags at 4°C.

1. Plant characters measured

Two selected categories of characters from the IBPGR/ICRISAT peanut (groundnut) descriptors (Descriptors for Groundnut, 1992) were studied: qualitative (morphological) and quantitative (agronomic) characters. They were scored with two to fifteen states and were analyzed. These parameters were measured in three replicates per accession (Table 2).

1.1 Oil content

To measure oil content in peanut accessions, oil was extracted using the Soxhlet apparatus (FOSS, Soxtec™ 8000), for each 1 g of kernel peanut placed on the thimbles, 25 ml of hexane was poured on the extraction vessels. The difference of vessel weight before and after extraction showed oil content. The oil content of each accession was measured in triplicate and results were expressed as the percentage of total oil content.

1.2. Storage protein extraction

Storage proteins were extracted from single crushed seeds according to U. K. Laemmli's (1970) protocol with minor modifications. After removing the skin, the peanut kernels were finely milled and defatted with hexane (10 ml/g dry weight) at -20°C overnight. 100 mg of dry peanut powder was mixed with 100 µl of fresh extraction solution, the mixture was incubated at 65°C for 30 min and then centrifuged 1 min at 10,000 g.

2. Data analysis

Quantitative morphological data were first subjected to descriptive analysis and ANOVA tests and the correlation matrix was performed on all quantitative characters to determine the relationships between them. The frequency table was used for a comparison of means by the analysis of variance (ANOVA) using Tukey's test ($p < 0.05$). The structure of morphological diversity was described using Principal Component Analysis (PCA), and the Hierarchical Ascendant Classification (CAH) was used to explore the relationship between

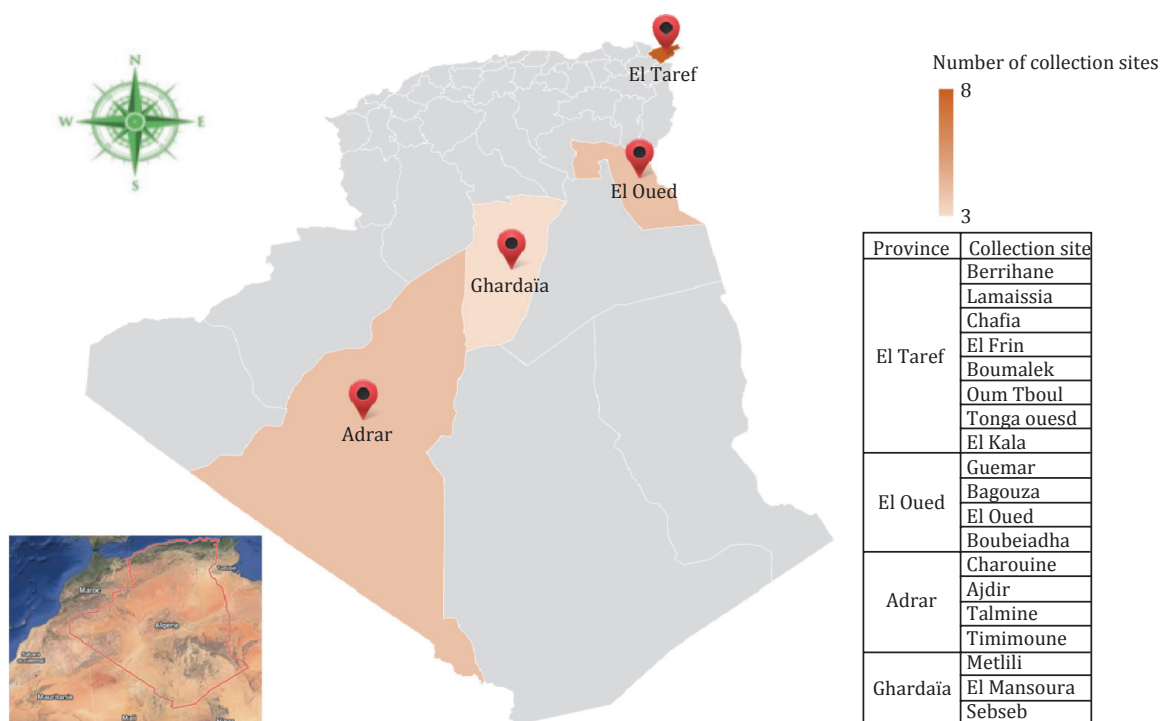


Fig. 1. Map of sampling locations for peanut accessions

Рис. 1. Карта мест сбора образцов арахиса

Table 1. Geographic origin of the studied Algerian peanut (*Arachis hypogaea* L.) accessions
Таблица 1. Географическое происхождение изученных алжирских образцов арахиса (*Arachis hypogaea* L.)

Geographic origin	Region where collected / location	Accessions name	Geographic zone	Bioclimatic floor
Taref	Berrihane, El Kala	Berrihane 1	Coastal	Subhumid
		Berrihane 2		
	Lamaissia, El Kala	Lamaissia 1		
		Lamaissia 2		
	Chefia, El Kala	Chefia 1		
		Chefia 2		
	El Kala	El Kala 1		
		El Kala 2		
		El Kala 3		
		El Kala 4		
	El Frin, El Kala	El Frin		
	Boumalek, El Kala	Boumalek		
Oum Tboul, El Kala	Oum Tboul			
Tonga-Ouest, El Kala	Tonga-Ouest			
Ghardaïa	Sebseb	Sebseb 1	Northern Sahara	Saharan
		Sebseb 2		
		Sebseb 3		
	El Mansoura	El Mansoura		
	Metlili	Metlili		
El Oued	Boubeiadha, Taghzout	Boubeiadha 1	Northern Sahara	Saharan
		Boubeiadha 2		
	Guemar	El Oued 1		
	Bagouza, Taghzout	El Oued 2		
	El Oued	El Oued 3		
		El Oued 4		
Adrar	Charouine	Hiba 1	Central Sahara	
		Hiba 2		
	Ajdir, Timimoune	Adrar 1		
	Talmine, Timimoune	Adrar 2		
	Talmine, Timimoune	Timimoune		

Table 2. Morphological and agronomic descriptors used for statistical analysis**Таблица 2. Морфологические и хозяйственно ценные признаки, использованные в статистическом анализе**

	Abbreviation	Character name	Character state	Range - Frequency (%) Average	
Morphological descriptors	Stem	Gr.hab	0 - Erect; 1 - Decumbent-1; 2 - Decumbent-2; 3 - Decumbent-3; 4 - Procumbent-2	0 - 46.67; 1 - 10.00; 2 - 3.33; 3 - 36.67; 4 - 3.33	
		Bra.pat	0 - Irregular without flowers on main stem; 1 - Irregular with flowers on main stem; 2 - Alternate; 3 - Sequential	0 - 3.33; 1 - 23.33; 2 - 13.33; 3 - 40.00	
		Num.bra	Number of branches	0 - Secondary; 1 - Tertiary	0 - 63.33; 1 - 36.67
		St.pig	Stem pigmentation	0 - Absent; 1 - Present	0 - 50.00; 1 - 50.00
		St.src	Stem surface	0 - Glabrous; 1 - Sub-glabrous, hairs in one or two rows along the main stem; 2 - Moderately hairy, three or four rows along the main stem; 3 - Very hairy, most of the stem surface covered with hairs; 4 - Woolly	0 - 30.00; 1 - 40.00; 2 - 6.67; 3 - 0.00; 4 - 3.33
		Bch.dty	Branching density	0 - Moderate; 1 - High	0 - 20.00; 1 - 80.00
	Flower	Inf.typ	Inflorescence type	0 - Simple; 1 - Complex	0 - 26.67; 1 - 73.33
		Peg.pig	Peg pigmentation	0 - Absent; 1 - Present	0 - 56.67; 1 - 43.33
		Std.pet.col	Standard petal color	0 - Yellow 9B; 1 - Orange 24A	0 - 66.67; 1 - 33.33
		Flo.dis	Flower distribution	0 - Alternate; 1 - Complex; 2 - Continuous	0 - 33.33; 1 - 20.00; 2 - 46.67
	Leaflet	Lflt.shp	Leaflet shape	0 - Oblong-lanceolate; 1 - Oblong; 2 - Obovate; 3 - Ovate; 4 - Suborbicular; 5 - Wide-elliptic; 6 - Narrow-elliptic; 7 - Oblong-elliptic; 8 - Elliptic; 9 - Cuneate	0 - 3.33; 1 - 6.67; 2 - 23.33; 3 - 6.67; 4 - 3.33; 5 - 6.67; 6 - 16.67; 7 - 13.33; 8 - 6.67; 9 - 13.33
		Lflt.src	Leaflet surface	0 - Almost glabrous on both surfaces; 1 - Almost glabrous above, hairs below; 2 - Almost glabrous above, hairs and/or bristles below	0 - 70.00; 1 - 20.00; 2 - 10.00
		Lflt.mgn	Leaflet margin	0 - Entire; 1 - Hairy	0 - 40.00; 1 - 60.00
		Lflt.tip	Leaflet tip	0 - Obtuse; 1 - Acute	0 - 56.67; 1 - 43.33
		Lef.col	Leaf color	0 - Green 129; 1 - Green 134A; 2 - Green 135; 3 - Green 140B; 4 - Green 142; 5 - Yellow-green 144C; 6 - Yellow-green 149	0 - 3.33; 1 - 13.33; 2 - 6.67; 3 - 13.33; 4 - 20.00; 5 - 26.67; 6 - 16.67
	Root	Nod.cap	Nodulation capacity	0 - Without nodules; 1 - With nodules	0 - 80.00; 1 - 20.00
Pod	Pod.csn	Pod constriction	0 - None; 1 - Slight; 2 - Moderate; 3 - Deep; 4 - Very deep	0 - 40.00; 1 - 13.33; 2 - 20.00; 3 - 20.00; 4 - 6.67	
	Txt.pod.src	Texture of pod surface	0 - Fine; 1 - Medium; 2 - Coarse	0 - 20.00; 1 - 13.33; 2 - 66.67	
	Pod.bk	Pod beak	0 - Absent; 1 - Slight; 2 - Moderate; 3 - Prominent; 4 - Very prominent	0 - 13.33; 1 - 20.00; 2 - 50.00; 3 - 3.33; 4 - 13.33	
	Pod.ret	Pod reticulation	0 - None; 1 - Slight; 2 - Moderate; 3 - Prominent; 4 - Very prominent	0 - 10.00; 1 - 36.67; 2 - 3.33; 3 - 23.33; 4 - 26.67	

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		Abbreviation	Character name	Character state	Range - Frequency (%) Average
Morphological descriptors	Seed	Num.sd.pod	Number of seeds per pod	0 - 2-1; 1 - 2-3-1/2-1-3; 2 - 3-2-1/3-1-2	0 - 73.33; 1 - 16.67; 2 - 10.00
		Teg.asp	Tegument aspect	0 - One color; 1 - Variegated	0 - 56.67; 1 - 43.33
		Sd.shp	Seed shape	0 - Round; 1 - Ovate; 2 - Oblong; 3 - Cylindric	0 - 10.00; 1 - 33.33 2 - 33.33; 3 - 23.33
		Teg.col	Tegument color	0 - Orange-yellow 6; 1 - Orange-yellow 19; 2 - Orange 26; 3 - Orange 28; 4 - Orange 29; 5 - Red-orange 31; 6 - Red-orange 33; 7 - Red-orange 34; 8 - Red-orange 35; 9 - Pink-red 37; 10 - Grey-orange 165; 11 - Grey-orange 172; 12 - Grey-orange 175; 13 - Grey-orange 177; 14 - Grey-orange 178	0 - 3.33; 1 - 6.67; 2 - 16.67; 3 - 3.33; 4 - 3.33; 5 - 6.67; 6 - 3.33; 7 - 10.00; 8 - 3.33; 9 - 3.33; 10 - 3.33; 11 - 6.67; 12 - 10.00; 13 - 13.33; 14 - 6.67
		Sd.col	Seed color	0 - Yellow 1; 1 - Yellow 4; 2 - Yellow 5; 3 - Yellow 8; 4 - Yellow 10; 5 - Yellow 13	0 - 3.33; 1 - 63.33; 2 - 3.33; 3 - 13.33; 4 - 10.00; 5 - 6.67
Agronomic descriptors	Stem	Pl.wdth	Plant width or Spread (cm)	Between 06 to 17	11.00
		Hght.stm	Height of main stem (cm)	Between 28 to 61	33.00
		Dys.mty	Days to maturity	0 - Between 80 to 100 days; 1 - Between 100 to 120 days; 2 - Between 120 to 140 days	0 - 56.67; 1 - 26.67; 2 - 16.67
	Flower	Dys.50%.fwg	Days to 50% flowering	0 - Between 20 and 45 days; 1 - Between 45 and 75 days	0 - 70.00; 1 - 30.00
	Leaflet	Lflt.lgth	Leaflet length (mm)	Between 19.08 to 59.35	40.27
		Lflt.wdth	Leaflet width (mm)	Between 10.44 to 31.45	21.01
		Lef.are	Leaf area (cm ²)	Between 4.29 to 11.22	6.93
		Chlo.ctn	Chlorophyll content (SPAD unit)	Between 27.40 to 49.9	22.50
	Pod	Pod.lgth	Pod length (mm)	Between 10.26 to 55.79	45.53
		Pod.wdth	Pod width (mm)	Between 5.62 to 20.13	14.51
		Wght.10 pods	Weight of 10 pods (gr)	Between 5.32 to 20.92	15.6
	Seed	Wght.100sd	Weight of 100 seeds (gr)	Between 30.37 to 108.4	78.03
		Sd.lgth	Seed length (mm)	Between 8.5 to 22.32	13.79
Sd.wdth		Seed width (mm)	Between 6.23 to 12.26	6.03	
Oil.ctn		Oil content (%)	Between 36.68 to 56.17	19.49	

the peanut accessions using arithmetic averages method (UPGMA); these analyses were carried out using data analysis software (XLSTAT 2016.02).

Concerning data analysis of storage proteins, electrophoresis profiles were analyzed using the Image Lab software version 5.1 (Biorad). The presence/absence of a band was scored as 1/0, respectively, and the employment of the Euclidean distance to the binary matrix served to calculate the pairwise distance among the accessions, which were clustered by the UPGMA method.

Results

1. Variation in morphological and agronomic characters

The frequency of appearance of morphological descriptors in the studied 30 accessions of *A. hypogaea* is presented in Table 2. The analysis of variance (ANOVA) showed a high significant variation ($p < 0.001$) among the peanut accessions for all of 13 agronomical characters assessed. Table 3 discloses a descriptive statistics estimation of all characters. The most variable ones included the weights of 10 pods and 100 seeds that ranged from 5.32 g to 20.92 g with a CV of 42.53%, and from 30.37 g to 108.4 g with a CV of 40.12%, respectively. These results can be attributed to the important variation observed in seed and pod sizes.

2. Relationships between agronomic characters

Pearson's correlation coefficients revealed significant associations among the evaluated different agronomic characters. Generally, stem architecture characters were significantly correlated with seed and pod characters. The highest significant correlation coefficients were noticed between leaflet length / leaflet width and the weight of 10 pods / seed width ($r = 0.751, p < 0.0001$, and $r = 0.776, p < 0.0001$, respectively). Furthermore, seed length and pod length were positively and significantly correlated ($r = 0.767, p < 0.0001$), hence any restriction of pod growth may cause smaller seeds. These results corroborated those reported by M. A. Zaman et al. (2011). A significant but weak correlation was also observed between leaflet length / plant width ($r = 0.456, p < 0.0001$), oil content / 10 pod weight ($r = 0.330, p < 0.0001$), and between the height of the main stem / pod width ($r = 0.302, p < 0.0001$) (see Table 3). Among leaf characters, leaf length was highly and significantly correlated with leaflet surface ($r = 0.553, p < 0.0001$), but did not correlate with leaf area. On the other hand, branching pattern was inversely correlated with seed length and the weight of 10 pods ($r = -0.594, p < 0.0001$, and $r = -0.554, p < 0.0001$, respectively). Slight correlations were observed between plant width / leaflet shape and growth habit / nodulation capacity ($r = 0.488, p < 0.0001$, and $r = 0.324, p < 0.0001$, respectively).

Table 3. Descriptive statistics estimation of agronomical characters assessed in the studied accessions

Таблица 3. Описательно-статистическая оценка хозяйственно ценных признаков у изучаемых образцов

Quantitative character	Min	Max	Mean \pm SE	CV%	<i>p</i>	Fisher effect size, %
Hght.stm	28.00	61.00	44.93 \pm 7.77	17.30	< 0.0001	13.32
Pl.wdth	6.00	17.00	9.69 \pm 2.62	26.98	< 0.0001	3.10
Lflt.lgth	19.08	59.35	42.27 \pm 8.21	19.43	< 0.0001	4.36
Lflt.wdth	10.44	31.45	21.88 \pm 4.21	19.23	< 0.0001	10.46
Chlo.ctn	27.40	49.90	39.48 \pm 5.23	13.25	< 0.0001	2.36
Lef.are	4.29	11.22	6.58 \pm 1.39	21.10	< 0.001	7.80
Pod.lgth	10.26	55.79	28.39 \pm 8.89	31.32	< 0.0001	16.52
Pod.wdth	5.62	20.13	13.41 \pm 3.06	22.84	< 0.0001	1.30
Wght.10 pod	5.32	20.92	11.98 \pm 5.09	42.53	< 0.0001	11.68
Wght.100 sd	30.37	108.40	53.05 \pm 21.29	40.12	< 0.0001	4.75
Sd.lgth	8.53	22.32	14.74 \pm 3.71	25.15	< 0.0001	9.17
Sd.wdth	6.23	12.26	8.59 \pm 1.38	16.06	< 0.0001	1.91
Oil.ctn	36.68	56.17	45.02 \pm 4.01	8.90	< 0.0001	13.29

Note: SE – standard error, CV% – coefficient of variation, *p* – probability value

Примечание: SE – стандартная ошибка, CV% – коэффициент вариации, *p* – уровень значимости

On the other hand, leaflet width and length revealed similar coefficients of variation among the studied accessions (19.23% and 19.43%, respectively) with an important positive correlation confirmed by the principal component analysis (Fig. 2, a). However, these characters; in addition to chlorophyll content, presented the least coefficients of variation (8.90% and 16.06%, respectively).

3. Principal Component Analysis

The relative importance of each character to explain the variation among the 30 *A. hypogaea* genotypes was assessed using the Principal Component Analysis (PCA). The first seven components explained 64.03% of the total variation among the 30 *A. hypogaea* accessions (Table 4). F1 separates accessions on the basis of three characters: branching pat-

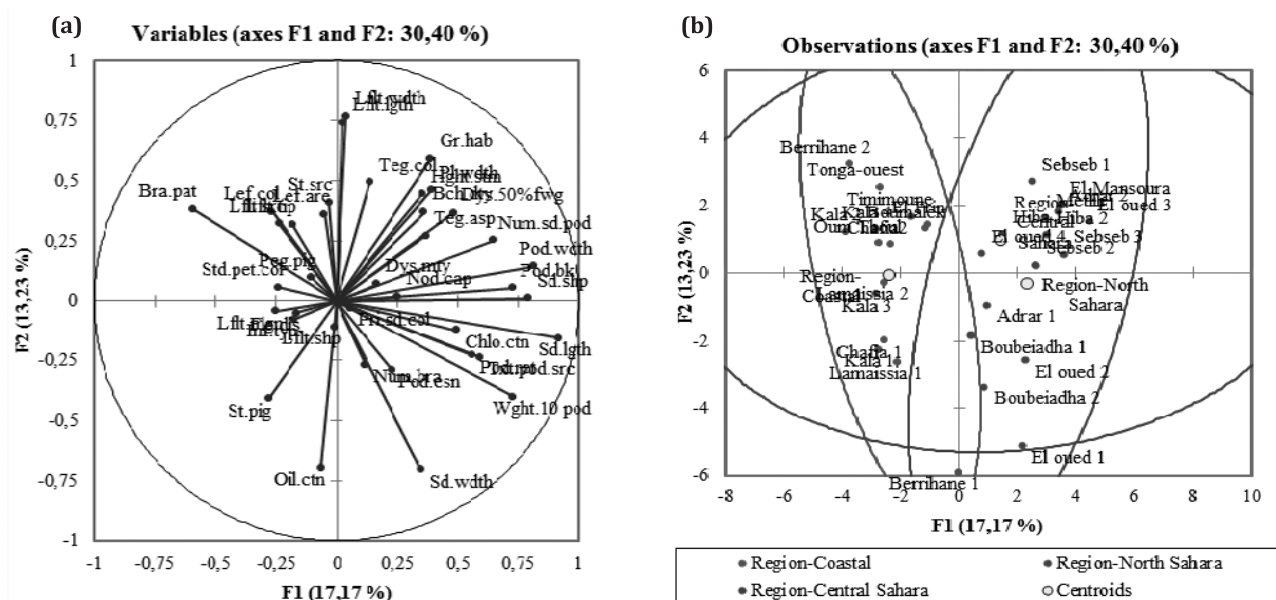


Fig 2. Principal Component Analysis of morphological characters: (a) diagnostic biplot, (b) graph of observations

Рис. 2. Анализ морфологических признаков методом главных компонент: (a) диагностическая диаграмма, (b) график наблюдений

Table 4. First seven principal components related to 38 characters studied in 30 *Arachis hypogaea* L. genotypes assessed

Таблица 4. Первые семь главных компонент для 38 изученных признаков у 30 проанализированных генотипов *Arachis hypogaea* L.

	Principal component axis						
	F1	F2	F3	F4	F5	F6	F7
Eigen value	6.526	5.028	3.071	2.834	2.770	2.297	1.805
Variability (%)	17.174	13.231	8.081	7.459	7.291	6.044	4.751
Cumulative %	17.174	30.404	38.485	45.944	53.235	59.279	64.030
Eigen vector loadings							
Gr.hab	0.152	0.264	0.161	-0.203	0.055	0.116	-0.117
Bra.pat	-0.232	0.171	0.040	-0.110	0.136	0.037	-0.164
Num.bra	0.046	-0.120	0.333	-0.060	-0.210	0.093	0.147
Hght.stm	0.138	0.198	-0.061	0.080	-0.027	-0.186	0.058
Pl.width	0.152	0.205	0.187	0.005	-0.073	0.060	-0.015
St.pig	-0.110	-0.184	0.205	0.082	0.133	0.353	0.014
St.src	-0.012	0.183	0.100	0.322	-0.131	0.186	0.036
Inf.typ	-0.068	-0.034	0.350	-0.193	0.024	0.115	0.125
Std.pet.col	-0,095	0.025	0.076	-0.053	-0.050	0.062	0.392
Peg.pig	-0.041	0.043	-0.331	-0.143	-0.243	0.138	0.048
Flo.dis	-0.065	-0.022	-0.224	0.037	-0.180	0.033	-0.326
Lef.col	-0.105	0.166	-0.182	0.024	0.174	0.002	0.190
Lfl.lgth	0.008	0.331	0.152	0.056	-0.062	0.201	-0.019
Lfl.width	0.015	0.343	0.079	0.064	0.081	0.057	-0.155

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	Principal component axis						
	F1	F2	F3	F4	F5	F6	F7
Lft.shp	-0.002	-0.048	-0.193	-0.028	0.061	0.402	0.172
Lft.src	-0.093	0.144	0.165	0.356	-0.042	0.144	-0.168
Lft.mgn	-0.099	-0.018	0.120	0.279	-0.228	0.024	-0.181
Lft.tip	-0.072	0.142	-0.083	-0.012	-0.309	0.000	-0.001
Dys.50%fwg	0.189	0.163	0.044	-0.052	0.136	-0.237	0.176
Dys.mty	0.065	0.032	-0.040	0.237	0.356	-0.125	0.160
Chlo.ctn	0.193	-0.054	0.135	-0.001	-0.177	-0.239	0.331
Bch.dty	0.141	0.165	0.252	0.145	-0.224	-0.128	0.034
Nod.cap	0.098	0.008	-0.043	0.096	-0.196	0.391	0.236
Lef.are	-0.019	0.162	0.021	-0.140	0.395	0.227	0.019
Pod.csn	0.089	-0.130	0.132	-0.281	-0.225	-0.033	-0.087
Num.sd.pod	0.253	0.112	0.096	-0.206	0.112	0.045	-0.014
Txt.pod.src	0.231	-0.105	-0.110	0.122	-0.012	0.026	-0.324
Pod.bk	0.284	0.024	0.059	-0.168	-0.125	-0.051	-0.082
Pod.ret	0.219	-0.103	-0.052	0.048	-0.039	0.300	0.015
Teg.asp	0.145	0.120	-0.184	-0.182	-0.114	0.052	-0.101
Teg.col	0.053	0.220	0.037	0.282	0.102	-0.150	0.024
Pri.sd.col	0.022	-0.013	-0.248	0.261	-0.133	-0.005	0.362
Oil.ctn	-0.025	-0.311	0.271	0.024	0.105	0.031	-0.030
Pod.wdth	0.319	0.065	-0.088	0.050	-0.028	0.092	-0.032
Wght.10 pod	0.285	-0.180	0.058	0.188	0.117	0.034	-0.077
Sd.wdth	0.136	-0.315	0.032	0.234	0.117	-0.044	-0.101
Sd.lgth	0.358	-0.070	-0.053	0.029	0.055	0.034	0.015

tern, seed length, and pod width. Concurrently, F2 separates accessions on the basis of four characters: leaflet width and length, seed width, and oil content. According to the study by S. T. Katzamanidis et al. (2006), the major botanical accessions of *A. hypogaea* were previously classified on the basis of leaf and stem morphological differences. PCA results also revealed that leaflet size (length and width), seed shape and size, oil content, and branching pattern were the principal characters to discriminate between the examined *A. hypogaea* accessions. On the other hand, characters which have a low loading in the distinction of accessions were growth habit and leaf color for F1, and peg pigmentation and days to maturity for F2.

These results agree with those reported by P. Mahalakshmi et al. (2005), Idi Garba et al. (2015), and F. B. Neya et al. (2017). Regarding the regional distribution on the graph of observations (see Fig. 2, b), two main groups can be distinguished. The first group is composed of accessions from the coastal region, while the second one of those from the northern Saharan region. A third small group can be observed, in-

corporating accessions from the central Sahara. The accessions from the coastal region were characterized by an alternate and sequential branching pattern, with flowers on the main stem, and small seed length and pod width. However, accessions from the northern Sahara presented an irregular branching pattern, without flowers on main stem. These accessions were characterized by high seed length and pod width. The third group consisted of accessions with more restricted distribution; accessions of this group were distinguished by a large size of leaflets and poor oil content.

4. Relationships among the studied accessions according to morphological characters

The relationships among the 30 *A. hypogaea* accessions based on the studied morphological characters were assessed using cluster analysis. The typology of the dendrogram clustered the 30 accessions into two major groups identified as I and II, using a dissimilarity index limit of 157.64 (Fig. 3). The number of genotypes per cluster varied from 5 in cluster II to 13 in cluster I. Cluster I represented 43.33% of the studied population, including 12 accessions originated from Taref and

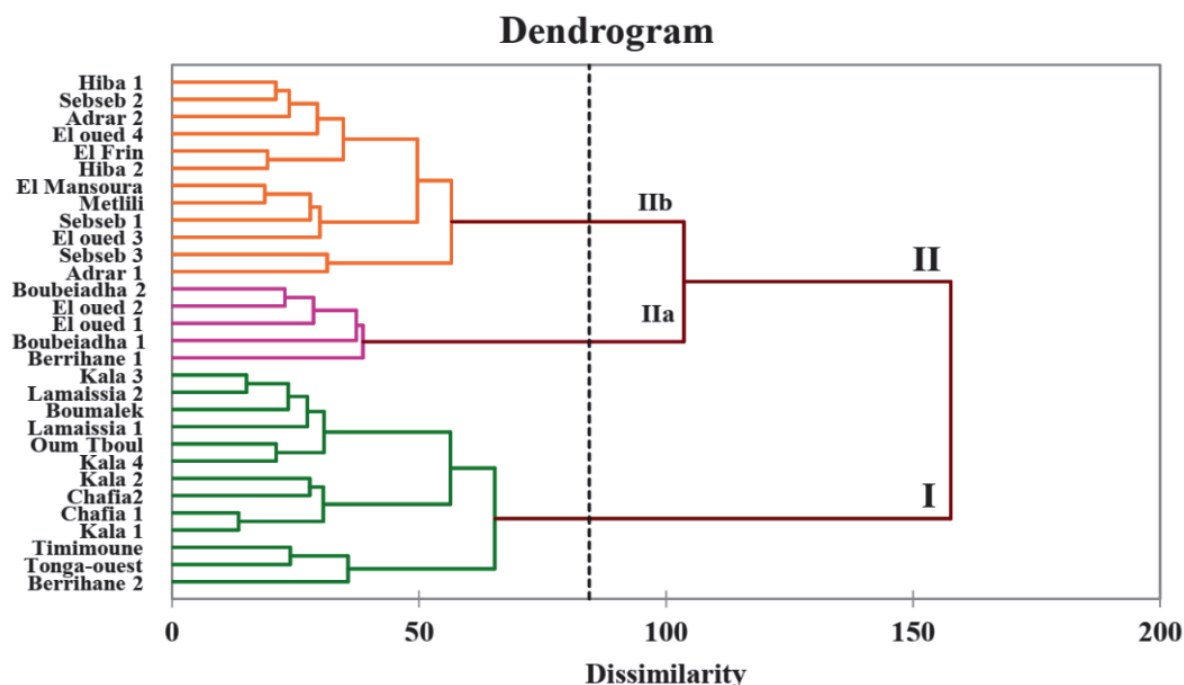


Fig. 3. Dendrogram based on the UPGMA method showing the relationships among the studied accessions

Рис. 3. Дендрограмма на основе метода UPGMA, показывающая взаимосвязи среди изучаемых образцов

the only Timimoune accession from Adrar (see Table 1). This cluster was the most distinguished and the remoter one, supposedly because of the particular subhumid climate in the region of Taref.

The second major cluster (II) grouped most of the accessions originated from the northern and central Saharan regions and corresponded to 56.67% of the total studied population; this cluster was divided into two subclusters, IIa and IIb. The first subcluster, IIa, contains five accessions, four of which were from El Oued, whereas one accession originated from Taref (see Fig. 3 and Table 1), representing 16.66% of the total studied population. Twelve accessions representing 40% of the total population, including those of different origin, namely Ghardaïa, Taref, El Oued, and Adrar, were distinctly grouped into subcluster IIb (see Fig. 3 and Table 1). This subcluster is the most heterogeneous among those identified; it is closer to subcluster IIa than the first one over a distance of 104.18. Except Berrihane 1 and El Frin, all accessions of the second cluster were cultivated in the Saharan climate. Clustering of these accessions in the same group can be explained by their adaptation to the specific climate of the Sahara.

5. Analysis of gel electrophoresis

In peanut, main seed storage proteins (about 87%) are globulins comprising two major fractions: arachin and conarachin described by X. Q. Liang et al. (2006). Total proteins extracted from thirty peanut accessions were separated by one-dimensional SDS-PAGE (Table 5), and the protein profiles revealed a few major quantitative and qualitative differences (Fig. 4); total bands distributed in the range of 250–6 kDa were scored. Electrophoresis profiles showed that different peanut accessions had different storage protein compositions generating 8 different genotypes presented in Figure 4.

The evaluation of band density using the Image Lab software version 5.1 (Biorad) revealed few differences among the studied accessions (data not shown). Most of the bands, including 4a, 3b, 4b, 1c, 2c, 3c, 4c, 1d and 2d, presented variable density on the electrophoresis profile. This variation may be due to the adaptation of accessions to ecogeographic factors,

soil richness in fertilizer like nitrogen, and the agronomic practices of the region (Chowdhury et al., 2015).

The number of protein bands present in each accession ranged from 23 to 30 bands. On the basis of the Rf value, the bands were grouped into four regions: region A (conarachin II), region B (acidic arachin), region C (basic arachin), and region D (conarachin I). No major qualitative variations were observed among the studied accessions in the arachin polypeptides (from 20 kDa to 50 kDa). The second fraction of globulin, represented by conarachin polypeptides, was divided into two groups with different molecular weight (> 50 kDa for conarachin II, and < 20 kDa for conarachin I).

Regarding the electrophoresis profiles, the studied peanut accessions presented similar conarachin II profiles (region A), characterized by a major protein of 61 kDa; this band corresponds to one of the important peanut allergens (Arah1).

In the electrophoresis profiles, 7 bands were identified in the region D, among which the first and the second conarachin I protein subunits were highly polymorphic; they presented a molecular weight of 20.2 kDa and 17.72 kDa, respectively, for most of the accessions, unlike 6 accessions of the collection from Taref and El Oued (Lamaissia 1, Lamaissia 2, Kala 3, El Oued 2, El Oued 3, and Boubeiadha 2) which missed these subunits. On the other hand, nine accessions, including, Chafia 1, Hiba 1, Sebseb 2, Metlili, El Mansoura, and others (see Fig. 4), presented these subunits with lower molecular weights (18.4 and 17.4, respectively). The accession Kala 4 had a specific profile and presented only the higher subunit (20.2 kDa).

6. Relationships among the studied accessions tested using storage protein separation

Cluster analysis of the accessions sorted the peanut population into three main groups at the distance of 56.8 (Fig. 5). Cluster I incorporated five accessions originated from Taref and Adrar, representing 16.66% of the total studied population. The second cluster represented 30% of the population and included 9 accessions of two different origins, Taref and Ghardaïa.

Table 5. Protein profiling of *Arachis hypogaea* L. accessions
Таблица 5. Профилирование белков у образцов *Arachis hypogaea* L.

Band number	Band name	Region	Molecular weight (kDa)	Relative to front
1	nl1	NL	250.0	0.051
2	nl2		238.9	0.067
3	nl3		181.2	0.190
4	nl4		174.7	0.212
5	nl5		116.0	0.337
6	nl6		110.5	0.353
7	a1	A	103.4	0.362
8	a2		94.6	0.394
9	a3		85.7	0.425
10	a4		78.2	0.441
11	a5		68.1	0.476
12	a6		61.0	0.525
13	a7		57.2	0.542
14	b1	B	54.6	0.583
15	b2		50.3	0.610
16	b3		44.5	0.629
17	b4		43.2	0.648
18	b5		40.5	0.673
19	b6		37.2	0.702
20	c1	C	35.0	0.735
21	c2		32.4	0.771
22	c3		29.5	0.783
23	c4		28.0	0.799
24	d1	D	20.2	0.821
			18.4	0.839
25	d2		17.72	0.850
			17.40	0.841
26	d3		14.74	0.873
27	d4		13.42	0.881
28	d5		11.32	0.908
29	d6	8.851	0.932	
30	d7	6	0.954	

Note: NI – non labelled

Примечание: NI – немаркированный

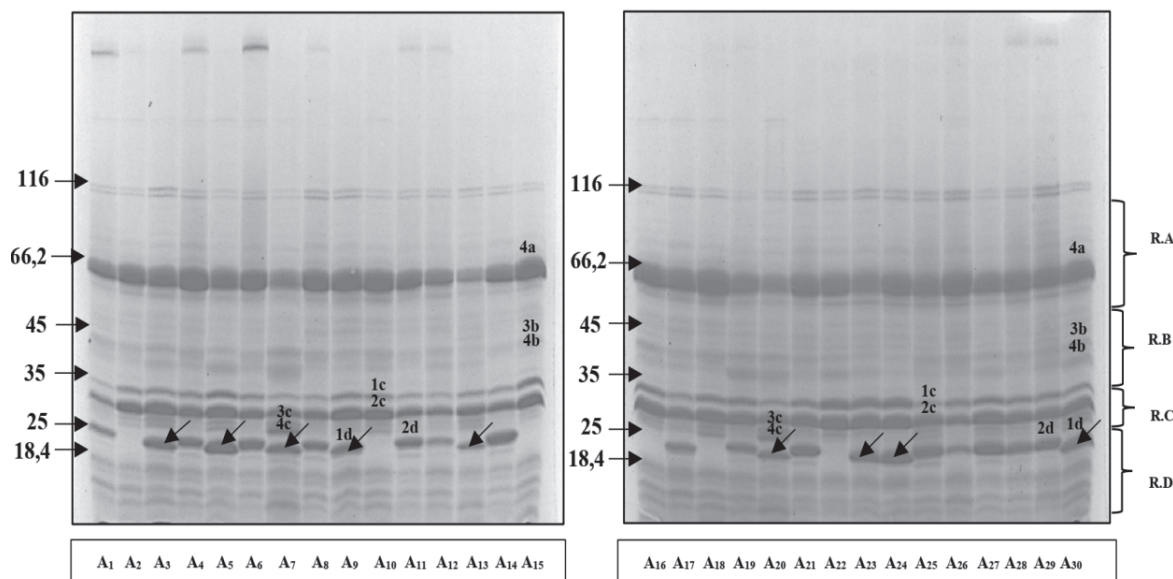


Fig. 4. Electrophoretic banding pattern generated by SDS-PAGE of *Arachis hypogaea* L. seed storage proteins:
A1 – Berrihane 1, **A2** – Lamaissia 1, **A3** – Sebseb 2, **A4** – Kala 1, **A5** – Chafia 1, **A6** – Boubeiadha 1, **A7** – Hiba 1, **A8** – Chafia 2, **A9** – Hiba 2, **A10** – Lamaissia 2, **A11** – Adrar 1, **A12** – Kala 2, **A13** – Sebseb 1, **A14** – El Oued 1, **A15** – Kala 3, **A16** – El Oued 2, **A17** – Adrar 2, **A18** – Boubeiadha 2, **A19** – El Frin, **A20** – El Oued 4, **A21** – Boumalek, **A22** – El Oued 3, **A23** – Metlili, **A24** – Sebseb 3, **A25** – Berrihane 2, **A26** – Kala 4, **A27** – Oum Tboul, **A28** – Tonga-ouest, **A29** – Timimoune, **A30** – El Mansoura, **R.A** – Region A, **R.B** – Region B, **R.C** – Region C, **R.D** – Region D, **R.NL** – Region non labelled

Рис. 4. Спектры электрофореза SDS-PAGE для запасных белков семян образцов *Arachis hypogaea* L.:
A1 – Berrihane 1, **A2** – Lamaissia 1, **A3** – Sebseb 2, **A4** – Kala 1, **A5** – Chafia 1, **A6** – Boubeiadha 1, **A7** – Hiba 1, **A8** – Chafia 2, **A9** – Hiba 2, **A10** – Lamaissia 2, **A11** – Adrar 1, **A12** – Kala 2, **A13** – Sebseb 1, **A14** – El Oued 1, **A15** – Kala 3, **A16** – El Oued 2, **A17** – Adrar 2, **A18** – Boubeiadha 2, **A19** – El Frin, **A20** – El Oued 4, **A21** – Boumalek, **A22** – El Oued 3, **A23** – Metlili, **A24** – Sebseb 3, **A25** – Berrihane 2, **A26** – Kala 4, **A27** – Oum Tboul, **A28** – Tonga-ouest, **A29** – Timimoune, **A30** – El Mansoura, **R.A** – область А, **R.B** – область В, **R.C** – область С, **R.D** – область D, **R.NL** – немаркированная область

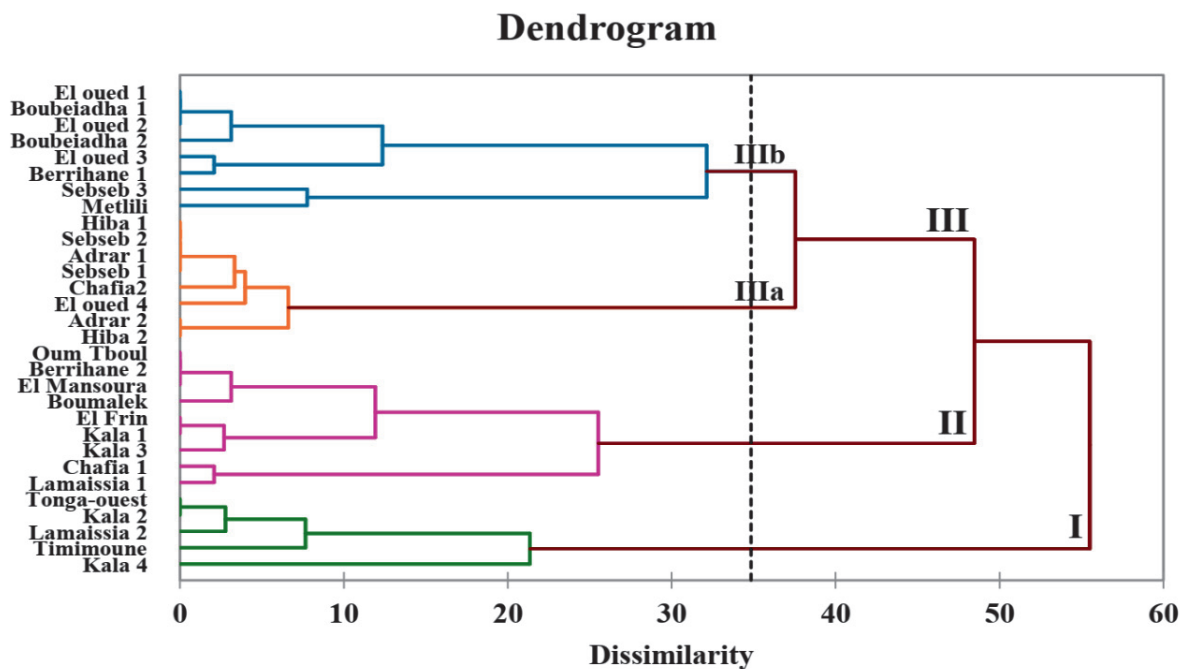


Fig. 5. Dendrogram generated using UPGMA cluster analysis based on storage seed proteins
Рис. 5. Дендрограмма, построенная с использованием кластерного анализа UPGMA на основе запасных белков семян

The third cluster was composed of two subclusters (IIIa and IIIb). The first subcluster contained genotypes from different geographic regions: 8 accessions, including Hiba 2, Adrar 2, Chafia 2, Sebseb 1, and others. This subcluster described 26.66% of the whole population studied. Similarly, the second subcluster harbored 8 accessions of different geographic origin, like El Oued and Ghardaïa. These subclusters are the closest ones at a distance of 38.21 and were the most heterogeneous, harboring accessions of different origin. Many authors investigated seed storage proteins in the genus *Arachis* (Singh et al., 1991; Liang et al., 2006). M. Jamalomidi et al. (2015) analyzed eleven accessions of peanut germplasm by SDS PAGE and showed that the diversity exists for seed storage protein profiles, but the germplasm was not well classified into subspecies or varieties. In contrast, an attempt was made to characterize 35 peanut cultivars raised by different pedigrees, using total soluble seed proteins separated by SDS-PAGE. Results showed a wide variation in the pattern of protein bands of the studied cultivars (Rao et al., 2013).

Discussion

To date, no study has assessed the variability of local peanut cultivars either at the morphological or at the molecular level. Hence, the current study is the first work where morphological characters were used to study the genetic diversity of *A. hypogaea* in Algeria. Diversity analysis using morphological characters is, therefore, very relevant for evaluation of genetic resources in the context of breeding programs.

The dendrogram describing the distances among accessions (see Fig. 2) revealed two distinguishes groups, clustering accessions originated from the coastal and Saharan regions. Generally, the studied Saharan accessions, including Ghardaïa, El Oued and Adrar, had high branching density and coarse texture of pod surface. This can be explained by the sunshine in the region and the type of sandy soil.

Branching in peanuts may produce a positive impact on yield, since the branches bear the leaves and also determine the solar radiation interception and utilization. In addition, the nodes of branches are potential sites for peg development and subsequent pod formation (Konlan et al., 2013). On the other hand, Saharan accessions, especially those of Ghardaïa, had the most significant height of main stems with the mean of 46.78 cm, compared with coastal accessions which were characterized by smaller stems (43.06 cm). These observations agree with those of G. S. Zhao et al. (2007), where soil texture was the major factor affecting bean growth; they found that plants in loam and sand were significantly larger than in clay (Lu et al., 2010).

Cylindric seed shape was a specific trait of all Ghardaïa accessions; seeds and pods of these accessions were consequently the longest ones in the collection. This can increase the chance to get pods with many seeds and enhance the yield. In fact, the Ghardaïa and Adrar accessions more frequently contained 3 seeds in their pods than 2 seeds, unlike the Taref accessions which rarely had pods with 3 seeds. Knowing that soil texture is an important measure of potential soil productivity (Lu et al., 2010), Zhao and collaborators (2015) revealed that the number of seeds in peanuts was shown to be the largest in the loam, large in the sand, and the smallest in the clay.

Soil texture also affects the nodulation capacity of peanut. Indeed, sand soil was the poorest in organic matter, like phosphorus and nitrogen (Zhao et al., 2015). Therefore, peanut roots were able to form a nitrogen-fixing symbiosis with bacteria from the genus *Rhizobium* using nodules developed fre-

quently on sand soils (Chevalier, 1934). Due to the soil texture, most of the Saharan accessions developed nodules on their root system. In contrast, all accessions originated from Taref produced glabrous roots. Except El Oued 03, the accessions originated from El Oued Province showed the best agronomic traits, thus being capable of improving peanut production because of their large seed size.

The El Oued accessions were the richest in oil content with an average of 48.31%. In addition to that, these accessions mature in 80 to 100 days, and 50% of their flowers open between 20 to 45 days after sowing. These characteristics make the accessions that originated from El Oued Province the perfect target for producers due to their large seed size, short period of maturation, and high oil content.

According to A. Krapovickas and W. C. Gregory (1994), branching pattern and growth habit are the most distinguishing traits between *A. hypogaea* subspecies, such as *A. hypogaea* subsp. *hypogaea* and *A. hypogaea* subsp. *fastigiata* Waldron. In fact, subsp. *hypogaea* was characterized by an irregular branching pattern without flowers on the main stem and a procumbent growth habit. However, subsp. *fastigiata* was characterized by the absence of flowers on the main stem with an erect growth habit. Proceeding from the results shown on the graph, accessions of the collection can be divided into two groups: accessions of the coastal region belonging to subsp. *fastigiata*, and those of Saharan region belonging to subsp. *hypogaea*.

Judging by the results obtained by A. R. Singh et al. (2018), seed protein fractions can serve as an important marker to assess the variability among germplasm and also distinguish between the subspecies and varieties. These results support those of PCA and confirm the presence of the two subspecies of *A. hypogaea* in the studied collection. We deduce also that conarachin I protein subunits play a crucial role in the discrimination of *A. hypogaea* subspecies.

A high correlation was observed between the classification based on agro-morphological traits and the one based on storage proteins. In fact, the two classification criteria grouped the accessions originated from Taref and El Oued into distinct groups. However, a heterogeneous group was observed for the two classifications. Besides that, the characters used for both classifications can distinguish between the *A. hypogaea* subspecies. Nevertheless, it is suggested that the number of accessions should be increased for better understanding the genetic relationships.

Conclusion

Genetic improvement of crops depends on the availability of genetic resources and their diversity. We report here for the first time on the genetic diversity of the Algerian peanut population analyzed using agro-morphological traits. The results showed a high genetic variability regarding agro-morphological traits and seed storage proteins in the studied collection. This diversity could mainly be attributed to the diverse agroclimatic conditions in the country. Hence, the three groups defined on the dendrogram based on agro-morphological characters belonged to two *A. hypogaea* subspecies and may represent the required variability for a basic collection. Results also show that the accessions from El Oued presented the best agro-morphological traits and can be highly recommended for cultivation and production of peanut by-products. With the aim to create an efficiency improvement program for Algerian peanut accessions, it is necessary to support this work with a genetic study to discriminate characters of agronomic importance.

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Прозрачность финансовой деятельности / The transparency of financial activities

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