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Association between Productivity, Fatty Acid Profiles, Oil Bodies' Ultrastructure and Molecular Markers in Peanut (*Arachis hypogaea* L.) Cultivars

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Abstract: The commercial Nigerian peanut cultivars Ex-Dakar, Samnut-22 and Samnut-26, the Egyptian cultivar Ismailia-1 and the American cultivar NC-7 were genetically differentiated and the cultivars' effects on growth and yield components, total lipid contents, including saturated and unsaturated fatty acids, and oil body size and number were investigated. Experiments were carried out at Cairo University Research Station, Giza, Egypt, during the summer seasons of 2016 and 2017. All cultivars had high contents of total unsaturated fatty acids and low contents of saturated fatty acids. Variations among tested cultivars in oil body size and number were documented and cultivars were ranked based on their oil bodies' size as Ismailia-1 > Samnut-26 > NC-7 > Ex-Dakar ≥ Samnut-22. Oil body size and number were inversely related. This variation can be explained by the different amounts of acidic amino acids and the protein structure on the surface of the oil bodies of these cultivars. The distributions of protein bodies and oil bodies seemed to be negatively correlated. Cultivar effect was significant, and variation was found in seed production and quality in addition to the size and number of oil bodies, oil productivity, and oil quality among cultivars. Seasonal effects were not evident due to the stability of the climatic conditions during the two seasons of the study.

Keywords: peanut; yield; quality traits; DNA extraction; RAPD; ISSR

1. Introduction

Peanuts are widely cultivated in the tropical and subtropical regions of the world. They are used in the form of seeds for human consumption and the haulm is used as fodder in the form of straw and hay for cattle. Peanut seeds can be eaten as snacks due to their many health benefits including a reduction in cardiovascular disease [1], colon, prostate, and breast cancer [2], osteoporosis [3] and diabetes [4]. They are a dietary source of calcium, magnesium, iron, zinc, phosphorus, vitamin E, riboflavin, thiamine and potash. For this reason, peanuts are used as a raw material in food and in the feed industry [5,6].

Genetic variation and relationships among genotypes or populations can be assessed using molecular markers. The efficiency of a marker technique in discriminating genotypes depends largely upon the polymorphisms detected among them. Cultivar differentiations have been documented in rice [7], broccoli and cauliflower [8], lettuce [9], banana [10], wheat [11], alfalfa [12] and tomato [13]

with different molecular marker systems. The evaluation of marker parameters such as Polymorphism Information Content (PIC), Marker index (MI), I and F in populations has highlighted the informative or discriminatory power of primer combinations in genetic studies [14,15]. Moreover, the markers are useful in genetic diversity estimation, phylogenetic studies, gene tagging, genome mapping and evolutionary biology in a wide range of plant species [9,16]. Ramadan et al. [17] evaluated the genetic diversity of six Egyptian fennel cultivars using Inter Simple Sequence Repeat (ISSR) and Start Codon Targeted (SCoT) markers. They found more discriminating results with the ISSR marker because it had the highest percentage of polymorphisms compared to the SCoT marker. They concluded that the ISSR marker can be reliable for genetic diversity evaluation among cultivars. Random Amplified Polymorphic DNA (RAPD) was also found to be an effective tool in detecting genetic diversity. Five peanut cultivars were genetically differentiated using twenty RAPD primers by Samaha et al. [18]. They found that RAPD markers were distributed in the peanut genome and helped to detect the genetic diversity of the peanut cultivars. They also concluded that the RAPD marker can be a significant tool in in peanut breeding programs. Phylogenetic relationships among ten Hordeum vulgare L. varieties were tested using RAPD-PCR and ISSR-PCR by El Atroush et al. [19]. RAPD-PCR showed a 41.77% polymorphism, while ISSR-PCR showed a 62.02% polymorphism. They concluded that both RAPD and ISSR markers would be suitable tools, but ISSR markers were more viable than RAPD in the detection of genetic variability among *Hordeum vulgare* (barley) varieties.

Peanuts are an important source of edible oil [2]. About 43 to 59 percent of the peanut seed weight is oil [20–23]. The peanut oil content and composition vary among different genotypes [21,23]. The quality of the oil depends on the composition of fatty acids, especially the proportion of unsaturated fatty acids (mainly oleic and linoleic acids) to saturated fatty acids [24]. Unsaturated fatty acids in peanut oil have antioxidant properties and help by lowering cholesterol and, as a result, reduce the heart disease risk [25,26]. The major components of peanut oil are oleic (25.1–35.2%), linoleic (8.70–13.03%), palmitic (3.77–4.53%), stearic acids (1.53–1.93%), arachidic and behenic acids (2.40–3.47%) [27]. Oil is stored as oil bodies (also known as lipid droplets) which are subcellular structures in the cytoplasm. Oil bodies are generally considered to be circular to ovoid in shape, with different diameters relevant to different cultivars and/or species within the range of 0.5–2.5 μm [28]. Oil bodies are filled with triacylglycerols (TAGs) enclosed by a monolayer of phospholipid (PL) embedded with integral membrane oleosin proteins [29]. The size and shape of oil bodies are affected by species and environmental factors [30,31]. It was found that local peanut cultivars comparable to internationally grown peanut varieties in Pakistan are more suitable for oil extraction and peanut butter development because of their high oleic acid to linoleic acid ratio [32]. Yusuf et al. [33] studied the fatty acid composition of peanut oil from different Ethiopian varieties. Varieties from warmer locations were better in oil quality than those from cooler climate locations. In the marker-assisted backcrossing–introgression lines over the locations, the most significant outcome was the stable expression of oil content, oleic acid, linoleic acid, and palmitic acid [34,35]. The peanut protein and oil body extraction by the aqueous enzyme extraction process may affect the yield of peanut protein and oil bodies by breaking down cell wall enzymes and degrading the main cell wall components. A peanut protein yield of 78.60% and oil body yield of 48.44% were achieved under the optimal condition [36]. The observation of the formation of oil bodies in the endosperm and embryo in Paeonia ostii seeds by transmission electron microscopy indicated that few oil bodies were scattered at the cell periphery in the endosperm, but oil bodies were numerous in the embryo [37]. This revealed that the ultrastructure of the endosperm and embryo significantly changed with the accumulation process of the oil bodies and fatty acid composition during the development [38].

Therefore, the objective of this study was to evaluate the conformity of the molecular marker system, viz. RAPD and ISSR, in assessing and analyzing the nature and the extent of genetic diversity among peanut cultivars and its effect on the size and number of oil bodies, seeds, oil productivity, and oil quality.

2. Materials and Methods

Five peanut cultivars were used in this study. The commercial Nigerian cultivars Ex-Dakar, Samnut-22 and Samnut-26 were obtained from the Institute for Agricultural Research (IAR), Samaru, Zaria, Nigeria. The Egyptian cultivar Ismailia-1 and the American cultivar NC-7 were obtained from the Agricultural Research Center (ARC), Giza, Egypt. The experiment was carried out at the Western farm of the Agricultural Experimental and Research Station, Faculty of Agriculture, Cairo University, Giza, Egypt (30°02′ N and 31°13′ E, altitude 22.5 m above sea level), during the summer seasons of 2016 and 2017. Monthly mean temperature and monthly relative humidity were recorded (Table 1). Monthly mean temperature values increased gradually from 28.90 and 29.27 °C in May to 30.47 and 31.37 °C in July and August in the 2016 and 2017 seasons, respectively. The maximum relative humidity was 46.33 during July and August in both years. There was no rain in the first season, while there was only 2 mm of rain recorded in July and September during the second season.

Table 1. Average temperature and relative humidity in the study area in Giza, Egypt, during the summer seasons of 2016 and 2017. Data obtained from the Central Laboratory for Agricultural Climate (CLAC), Agricultural Research Center (ARC), Egypt.

Month	2016			2017		
	Temperature (°C)	Relative Humidity (%)	Rainfall (mm)	Temperature (°C)	Relative Humidity (%)	Rainfall (mm)
May	28.90	38.67	0.00	29.27	34.00	0.00
June	30.57	31.67	0.00	29.33	35.33	0.00
July	30.45	46.33	0.00	31.37	42.33	2.00
August	30.47	44.33	0.00	30.85	46.33	0.00
September	28.85	44.33	0.00	28.60	46.00	2.00

A randomized complete block design (RCBD) with three replications was used with plots 9.6 m^2 and 0.6 m apart (two rows per plot). Soil samples were collected and tested in the lab. The soil was silty clay containing 3.4% sand, 47.1% silt, and 49.5% clay. The soil pH was 7.6 and electrical conductivity (EC) was 2.35 dSm^{-1}. Cultural practices were conducted according to the recommendation of the Agricultural Research Center (ARC), Egyptian Ministry of Agriculture.

Seeding hills were prepared 10 cm apart, and seeds were sown manually on April 26 in both years. Seedlings were thinned to keep only two seedlings per hill to avoid competition and poor growth (18–20 plants per plot). Flood irrigation was used. Inorganic fertilizers were added and used in the form of superphosphate (15% P_2O_5) at a rate of 480 kg/ha before seeding. Potassium sulfate (48% K_2O) at a rate of 240 kg/ha was added in 2 separate doses, the first one was added after the emergence and the second dose was added before flowering. Nitrogen was added at a rate of 72 units per hectare in the form of calcium nitrate (15% N). It was amended in two separate doses, one after emergence and the second three weeks later. A mixture of micronutrients, viz. boron, zinc, iron, and manganese, was sprayed three times as a foliar application after full emergence (40-day age) at 3-week intervals. Hoeing and hand pulling were relied upon in this experiment to control annual summer and perennial weeds. The experimental plots were maintained free of weeds throughout the growing season by hoeing four times at 20, 40, 60 and 80 days after sowing.

Data were collected on growth and yield components by randomly selecting five plants from the two inner ridges of each plot at harvest time to record plant height (cm), the number of branches/plant, the number of mature pods/plant, the weight of pods/plant and the weight of seeds/plant in grams. The quality characteristics of pods and seeds were assessed by determining the pod index (the weight of 100 pods in grams), seed index (the weight of 100seeds in grams), pod percentage (%) and shelling percentage (%). Biological yield (ton ha^{-1}), pod yield (kg ha^{-1}), seed yield (kg ha^{-1}) and oil yield (kg ha^{-1}) were assessed.

The total lipid content in peanut seeds for different cultivars was determined according to A.O.A.C [39]. Five grams of seeds were ground, and oil was extracted with petroleum ether (40–60 °C)

using Soxhlet apparatus. Lipids obtained were converted into fatty acid methyl esters (FAMEs) according to Rozes et al. [40], where 100 µL of each oil sample was placed in a test tube, 0.5 mL hexane was added to the oil, then 0.5 mL sodium methoxide solution (CH_3ONa) in anhydrous methanol (CH_3OH) 0.4 N was added and mixed, then allowed to stand for 1 min. The upper phase (hexane layer) was removed after decanting and was injected onto the chromatograph. The GC-MS system (Agilent Technologies, Santa Clara, CA, USA), equipped with a gas chromatograph (7890B) and a mass spectrometer detector (5977A) with a HP-5MS column (30 m × 0.25 mm internal diameter and 0.25 µm film thickness), was used at the Central Laboratories Network, National Research Centre, Cairo, Egypt. Helium was used as a carrier gas at a flow rate of 1.0 mL/min at a split ratio of 1:30. One µL of sample was injected and the temperature was programmed to remain at 150 °C for 1 min, then to rise by 2 °C /min, up to 200 °C, then to remain at this temperature for 4 min. After the 4 min period, the temperature rose again by 5 °C/min, up to 300 °C, then remained at this temperature for 1 min. The injector and detector were held at 280 °C and 300 °C, respectively. The mass spectra were obtained by electron ionization (EI) at 70 eV and using a spectral range of *m*/*z* 40–450 and a solvent delay of 2 min. The identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and The National Institute of Standards and Technology (NIST)Mass Spectral Library data.

2.1. DNA Extraction, Primers and DNA Amplification

Genomic DNA was extracted from a small amount of young and fresh leaves (0.1 g) collected from the cultivars using the Biospain Plant Genomic DNA Extraction Kit (BioFlux). Twelve primers (Table 2) obtained from Pharmacia Biotech (Amersham Pharmacia Biotech UK Limited, England, HP79 NA) were used for the identification of different peanut genotypes.

Table 2. Primers used and their annealing temperatures.

ISSR Primer	Sequence of Primer (5′–3′)	RAPD Primer	Sequence of Primer (5′–3′)
UBC-810	GAGAGAGAGAGAGAGAT	OP-B15	GGAGGGTGTT
UBC-812	GAGAGAGAGAGAGAGAA	OP-B18	CCACAGCAG
UBC-814	CTCTCTCTCTCTCTCTA	RMN-P1	CAGAAGCGGA
UBC-815	CTCTCTCTCTCTCTCTG	RMN-P3	TGTCATCCCC
UBC-822	TCTCTCTCTCTCTCTCA	UBC-402	CCCGCCGTTG
UBC-845	CTCTCTCTCTCTCTCTRG	UBC-475	CTCTCTCTCTCTCTCTRG

PCR reaction test was performed according to Weising et al. [41]. In total, 25-µL volume reaction mixture was used, containing: 5 µL of 5X green Taq DNA polymerase buffer, 100 µM of primer, 20 ng of total cellular DNA, 10 mM of each of dNTP, 25 mM $MgCl_2$, 5 U/µL of GoTaq DNA polymerase (Promega), up to 25 µL by nuclease-free water. PCR was performed in a DNA thermo cycler (BioRad T100, Foster City, CA, USA) and subjected to 35 cycles of the followings (45 s at 94 °C, 45 s at 37 °C, 1.5 min at 72 °C).

Band Scoring and Analysis

Polymorphism was scored on a presence or absence basis and data were analyzed using GenAlEx [42]. The analysis of data was performed using the diploid binary data criterion (1, present and 0, absent) that can distinguish each band position that corresponds to a Hardy–Weinberg Equilibrium (q), $q = (1 - band\ freq.)^{0.5}$ and $=1 - q$. For comparisons, heterozygosity was estimated differently [43,44]. Cluster analyses were based on similarity matrices using the unweighed pair group method arithmetic average (UPGMA) and relationships between cultivars were visualized as dendrograms. Differences between dendrograms were tested by generating cophenetic values for each dendrogram and via the assembly of a cophenetic matrix for each marker type.

For a genetic marker, the number of effective bands (*Ne*) was estimated according to the formula $Ne = 1 \div (p^2 + q^2)$, whereas expected heterozygosity (*He*) was calculated from the sum of the squares of

band frequencies ($He = 2 \times p \times q$; [45]), where Pi is the band frequency for the i-th band. Therefore, unbiased expected heterozygosity was estimated by the following equation: $uHe = (2N \div (2N - 1) \times He)$. Shannon's Information Index was calculated by the formula $I = -1 \times (p \times Ln(p) + q \times Ln(q))$.

2.2. The Transmission Electron Microscopy (TEM)

The seeds of the tested cultivars were fixed for 2 h in 2% (*w*/*v*) paraformaldehyde and 2.5% (*v*/*v*) glutaraldehyde in phosphate buffer 0.1 M, with a pH of 7.4. Following fixation, the seeds were washed with the phosphate buffer for 10 min, re-suspended in 1% (*w*/*v*) paraformaldehyde for 2 h, and rinsed three times with the phosphate buffer. Seeds were post fixed for 2 h with 1% (*w*/*v*) osmium tetroxide containing 0.8% (*w*/*v*) potassium hexacyanoferrate, prepared in phosphate buffer, followed by four washes with deionized water and sequential dehydration in acetone. All procedures were performed at 4 °C. Samples were embedded in Spurr's Resin and polymerized at 60 °C for 48 h. Ultra -thin sections (70 nm) were obtained with a diamond knife (45°, Diatome). Sections were stained with uranyl acetate and Reynolds lead citrate for 10 min and examined with a JEOL 1400 transmission electron microscope equipped with a Gatan Ultrascan ES1000 CCD Camera (Gatan Inc., USA). Embryos from three individuals were used in each analysis. Grids were stained with uranyl acetate and Reynolds lead citrate solutions. Samples were viewed on a JEM-1400 transmission electron microscope (JEOL, Tokyo, Japan).

Size Measurements of Oil Bodies in the Peanut Cell

In order to determine the oil body diameters, 80–140 measurements of oil body diameter and 15–20 measurements of protein body diameter were taken from each image (10 images per sample type) obtained from TEM at the same magnification (5 µm or 6000×) by a Nano Measurer 1.2. The determination of the mean diameter of the oil bodies and protein bodies was based on the results of 10 images.

2.3. Statistical Analysis

Data were statistically analyzed according to the procedures outlined by Steel et al. [46] using the MSTAT-C program [47]. Two-way ANOVA was used to test cultivar and year effects and their interactions. Means were compared and separated using the least significant difference test (Fisher's LSD) at a 5% level of probability. Regression analysis was performed to test the association between oil body number and size.

3. Results and Discussion

3.1. Agronomic Traits

3.1.1. Growth and Yield Components

The analysis of variance indicated no significant differences between the two years of the study, nor the interaction between years and cultivars. Hence, data for the two years were pooled together. Significant differences among cultivars in relation to the tested agronomic traits were evident (Table 3).

Significant differences among cultivars in all growth and yield parameters were observed (Table 4). Samnut-26 and Ex-Dakar cultivars achieved a higher plant height (56.33 and 53.75 cm, respectively), and there was no significant difference between them. The Ismailia-1 cultivar had the greatest number of branches per plant (6.17). NC-7 and Ismailia-1 cultivars achieved the highest number of pods per plant (130.50 and 121.50, respectively) and the highest pod weight per plant (40.85 and 39.73 g, respectively). The NC-7 cultivar had the highest seed weight per plant (27.65 g), followed by Samnut-22 and Ismailia-1 (25.90 and 25.50 g, respectively).

Table 3. Combined mean squares and treatments of the studied traits.

SOV	df	Growth and Yield Components				
		Plant Height, cm	Branches Number Plant^{-1}	Pods Number Plant^{-1}	Pods Weight Plant^{-1}, g	Seeds Weight Plant^{-1}
Year (Y)	1	760.033	16.133	1502.376	14.283	9.747
Error	4	7.783	0.933	353.916	3.254	3.418
Cultivar (V)	4	786.029 **	4.383 **	9136.385 **	158.885 **	77.612 *
Y × C	4	31.554	2.550	110.785	3.039	0.285
Error	16	21.617	0.642	377.400	1.589	2.694
SOV	**df**	**Quality traits**				
		Pod index, g	**Seed index, g**	**Shelling %**	**Pops %**	**Oil %**
Year (Y)	1	136.960	149.187	42.008	1.224	2.730
Error	4	21.383	5.941	5.413	0.009	0.368
Cultivar (V)	4	4314.896 *	955.454 **	44.208 **	32.003 **	41.965 **
Y × C	4	41.186	23.999	2.498	1.016	0.349
Error	16	28.994	5.807	9.408	0.464	1.918
SOV	**df**	**Peanut yields**				
		Biological yield, ton ha^{-1}	**Pod yield, kg ha^{-1}**	**Seed yield, kg ha^{-1}**	**Oil yield, kg ha^{-1}**	
Year (Y)	1	36.741	342,667.969	308,225.570	72,862.451	
Error	4	0.854	33,808.594	14,024.029	2578.194	
Cultivar (V)	4	20.436 **	9,026,173.828 **	4,257,374.732 **	963,607.223 **	
Y × C	4	2.895	67,365.234	52,818.314	11,728.743	
Error	16	3.010	18,486.328	14,078.038	3225.668	

* and ** indicate significance at 5% and 1% probability level, respectively.

3.1.2. Quality Traits

The results indicated a significant difference among cultivars in terms of seed index, shelling percentage, oil content percentage, pops percentage and pod index (Table 4). NC-7 and Ismailia-1 cultivars had the highest values of seed index (60.08 and 55.90 g, respectively), shelling percentage (65.82 and 64.52%, respectively) and oil percentage (44.63 and 46.85%, respectively) and the lowest values of pops percentage (2.12 and 2.51%, respectively). The Samnut-22 cultivar achieved the highest value of pod index (188.20 g) followed by Samnut-26 (171.90 g).

3.1.3. Yields

Significant differences among cultivars in biological yield, pod yield, seed yield and oil yield were observed (Table 4). NC-7 and Ismailia-1 cultivars had the greatest pod yield (1414 and 3572 kg ha^{-1}, respectively), the greatest seed yield (2729 and 2307 kg ha^{-1}, respectively) and the greatest oil yield (1219 and 1082 kg ha^{-1}, respectively). Samnut-22 achieved the highest biological yield (12.39 tons ha^{-1}).

In agreement with our findings, Abdel-Motagally et al. [48] found significant variations among Egyptian peanut cultivars in terms of yield components and quality traits and Iledun et al. [49] reported variations among Nigerian cultivars in terms of biomass yields, pod yield/ha, effective pods, 100-seed weight and seed yield/ha. Shams El-Din and Ali [50] found significant differences among Egyptian peanut cultivars in terms of the shelling percentage, as well as oil and protein yields. Tarawali [51] recommended the Samnut 22 cultivar for better seed and fodder production. The variation among peanut cultivars in yield components and quality can be explained by the genetic variation among cultivars, along with the environmental conditions [52]. Variations in oil content and oil composition among peanut cultivars were explained by the variation in the environmental conditions, the genetic structure and their interactions [20,53–55]. There was no drastic change in climatic conditions between the summer season of 2016 and that of 2017 (Table 1) and, as a result, there was no significant difference between the two seasons in relation to quality traits.

Table 4. Mean of studied traits of peanut treatments tested at field experiments in Giza, Egypt—combined data from 2016 and 2017 seasons.

Traits	Cultivars				
	Ismailia-1	**Ex-Dakar**	**Samnut-22**	**Samnut-26**	**NC-7**
Growth and yield components					
Plant height, cm	45.50b †	53.75a	27.33c	56.33a	42.42b
Plant branch number^{-1}	6.17a	4.33b	4.33b	4.83b	4.33b
Plant pod number^{-1}	121.50a	65.83b	51.12b	50.67b	130.50a
Plant pod weight^{-1}, g	39.73a	27.88d	37.83b	35.75c	40.85a
Plant seed weight^{-1}, g	25.50b	18.37d	25.90a	23.07c	27.65a
Quality traits					
Pod index, g	137.7c	122.1d	188.2a	171.9b	143.7c
Seed index, g	55.90b	33.93d	36.40c	35.17c	60.08a
Shelling %	64.52a	58.97ab	62.62a	61.10ab	65.82a
Pops %	2.51c	7.85a	4.27b	5.19b	2.12c
Oil %	46.85a	43.97b	39.75c	42.41bc	44.63b
Peanut yields					
Biological yield, ton ha^{-1}	11.18a	8.09b	12.79a	11.34a	12.39a
Pod yield, kg ha^{-1}	3572b	1287d	1825c	1988c	4141a
Seed yield, kg ha^{-1}	2307b	757.0d	1144c	1213c	2729a
Oil yield, kg ha^{-1}	1082.0b	332.2d	454.8c	514.9c	1219.0a

† Values followed by the same letters within a row for each cultivar are not significantly different ($p = 0.05$) based on a least significant difference test (Fisher's LSD) test.

3.2. Fatty Acid Profiles of Seed Oil

The quality of the oil fraction varies considerably among cultivars. The oil fraction quality depends on the fatty acid composition (Table 5), especially the proportion of unsaturated fatty acids—mainly oleic, linoleic and linolenic acids [24].

The major components of peanut oil were oleic, linoleic, palmitic, stearic and behenic acids in all cultivars (Table 5). Looking at the unsaturated fatty acids, oleic acid (C18:1) content ranged from 39.19 to 44.98%, linoleic acid content (C18:2) ranged from 31.65 to 34.60%, and the contents of saturated fatty acids such as palmitic (C16:0), stearic (C18: 0) and behenic acid (C22:0) were lower in all cultivars compared with the unsaturated fatty acids (oleic and linoleic acids). Palmitic acid content ranged from 12.07 to 14.54%, Stearic acid content ranged from 2.69 to 4.1%, and behenic acid content ranged from 3.22 to 4.31%. All cultivars had higher contents of unsaturated fatty acids (74.59% to 77.44%) compared to their saturated fatty acid content (22.25% to 24.74%).

The analysis of variance indicated a significant difference among cultivars in terms of fatty acid contents. The Ismailia-1 cultivar had the highest content of oleic acid (44.98%), followed by Samnut-26 (41.78%), Samnut-22 (41.25%), NC-7 (40.71%), and Ex-Dakar which had the lowest content (39.19%). The cultivars Ex-Dakar, Samnut-22 and NC-7 had almost similar relatively high contents of linoleic acid (34.6%, 34.09% and 34.12%), respectively), followed by Samnut-26 and Ismailia-1 (32.54% and 31.65%). Overall, the cultivar Ismailia-1 produced the highest amount of unsaturated fatty acids (77.74%), followed by Samnut-22 (77.34%). The highest contents of palmitic and stearic acids were observed in the cultivars Ex-Dakar and Samnut-22, while the lowest contents were found in the cultivars NC-7 and Ismailia-1. The highest content of behenic acid was found in the cultivar Samnut-22 (4.31%) and the lowest was found in Ismailia-1 (3.22%). The highest content of Lignoceric acid was achieved by Samnut-22 (1.96%), followed by NC-7 (1.85%), Ex-Dakar (1.31%), Ismailia-1 (1.26%) and Samnut-26 (1.16%). Overall, the cultivar Ismailia-1 produced the lowest amount of saturated fatty acids (22.25%), followed by Samnut-22 (22.65%) while the cultivar Ex- Dakar produced the highest amount of saturated fatty acids (25.4%).

Table 5. Mean of fatty acids composition of seed oils produced by the cultivars tested at field experiments in Giza, Egypt—combined data from 2016 and 2017 seasons.

Scientific Name of Fatty Acids	(Cxx:y)	Cultivars				
		Ismailia-1	Ex-Dakar	Samnut-22	Samnut-26	NC-7
		Fatty Acids%				
Oleic acid	C18:1	44.98a †	39.19d	41.25b	41.78b	40.71c
Linoleic acid	C18:2	31.65c	34.60a	34.09a	32.54b	34.12a
11-Eicosenoic acid	C20:1	1.11c	0.80d	2.00a	0.95cd	1.60b
Total unsaturated acid		77.74a	74.59d	77.34a	75.27cd	76.43b
Palmitic acid	C16:0	13.12b	14.54a	12.07c	14.03a	12.20c
Stearic acid	C18:0	3.20c	4.02a	2.69d	4.10a	3.64b
Arachidic acid	C20:0	1.45b	1.58a	1.62a	1.24c	1.69a
Behenic acid	C22:0	3.22d	3.95b	4.31a	3.71c	3.91b
Lignoceric acid	C24:0	1.26b	1.31b	1.96a	1.16b	1.85a
Total saturated acid		22.25c	25.4a	22.65c	24.74a	23.29b

† Values followed by the same letters within a row for each cultivar are not significantly different ($p = 0.05$) based on Fisher's LSD test.

In general, all cultivars were found to have a high content of total unsaturated fatty acids and a low content of saturated fatty acids. This indicates the importance of peanut seeds in human nutrition. The differences between peanut cultivars were also reported by Bell et al. [56], Anderson et al. [53], Ishag [57], Weiss [58], Ozcan and Seven [27], Hassan et al. [59], Carrin and Carelli, [23] and Manaf et al. [60]. Additionally, high unsaturated fatty acid levels provide antioxidant properties and support several metabolic processes [28]. Variations among cultivars could be attributed to the genotypic as well as the phenotypic characters of each cultivar and the variations in their branching patterns [6].

3.3. Genetic Variations Revealed by RAPD and ISSR Analysis

The RAPD analysis revealed a total of 30amplified fragments (ranging from four to 10 bands) with 46.7% polymorphism. The polymorphism of primers OP-B18 and RMN-P1 was equal (37.5%), while that of primer RMN-P3 was 75% (Table 6). ISSR primers showed a total of 36 amplified fragments (ranging from four to seven bands) with a polymorphism of 28.9%. The polymorphism ranged from 0.0% in the primer UBC-810 to 60% in both the UBC-812 and UBC-814 primers. The Polymorphism Information Content (PIC) was used to evaluate the informative potential of markers in different accessions and germplasms [14,15,61,62]. The average PIC for RAPD was 0.21, while that of ISSR was 0.19. With the primer RMN-P3, the PIC of RAPD was 0.37 and the minimum PIC value of 0.15 was recorded with the OP-B18 primer with ISSR; the highest PIC record was 0.30 for primers UBC-812 and UBC-814, while the lowest was 0.0 for the primer UBC-810, with an average of 0.25. Ramadan et al. [17] found more discriminating results with the ISSR marker than the SCoT marker among fennel cultivars. RAPD was also found to be an effective tool in detecting genetic diversity in peanut cultivars [18]. El Atroush et al. [19] compared RAPD and ISSR's efficacy and found that both RAPD and ISSR markers are suitable tools, but ISSR is a more viable marker than RAPD in the detection of the genetic variability among Hordeum vulgare (barley) varieties.

Table 6. Details of marker parameters calculated for RAPD and ISSR primers.

Primer	Size (bp)	Tb	Ub	Mb	Pb	P%	Freq.	Nb	Ne	I	He	uHe
RAPD PCR analysis												
OP-B18	169-2332	8	1	5	3	37.5	0.850	1.37	1.27	0.21	0.15	0.16
OP-B15	123-2499	10	0	5	5	50	0.820	1.50	1.40	0.31	0.22	0.24
RMN-P1	235-2015	8	0	5	3	37.5	0.875	1.37	1.31	0.24	0.17	0.19
RMN-P3	293-1056	4	0	1	3	75	0.850	1.75	1.73	0.52	0.37	0.41
Total	123-2499	30	1	16	14	46.7						
Mean		7.5	0.25	4	3.5		0.849	1.47	1.39	0.29	0.21	0.23
ISSR-PCR analysis												
UBC-402	220-932	4	0	3	1	25	0.95	1.25	1.24	0.17	0.12	0.14
UBC-810	123-768	5	0	5	0	0	1.00	1.00	1.00	0.00	0.00	0.00
UBC-812	97-1351	5	0	2	3	60	0.88	1.60	1.59	0.41	0.30	0.33
UBC-814	42-1196	5	0	2	3	60	0.88	1.60	1.59	0.41	0.30	0.33
UBC-815	160-1147	5	0	3	2	40	0.84	1.40	1.30	0.24	0.17	0.19
UBC-822	78-896	7	0	3	4	57.14	0.80	1.57	1.51	0.38	0.27	0.30
UBC-845	160-1147	5	1	3	1	20	0.76	1.40	1.22	0.20	0.13	0.15
Total	78-1196	36	1	21	14	28.9						
Mean		5.14	0.14	3	2		0.87	1.42	1.36	0.27	0.19	0.21

Tb = total number of al bands; Ub = unique bands; Mb = monomorphic bands; Pb = polymorphic bands; P% = polymorphism percentage; Nb = number of bands of each primer; Ne = number of effective bands; I = Shannon's informative index; He = expected heterozygosity; *uHe* = unbiased expected heterozygosity.

The unbiased heterozygosity (uH_e) ranged from 0.41 with the RMN-P3 primer to 0.16 with the primer OP-B18, with a mean record of 0.23 with ISSR; the uH_e ranged from 0.0 with the UBC-810 primer to 0.33 with the UBC-812 and UBC-814 primers, with an average of 0.21.

The Polymorphism Information Content (PIC) was classified into three classes [63]. The highly informative markers achieve PIC values > 0.50, the reasonably informative markers have PIC values between 0.25 and 0.50 and the slightly informative markers have PIC values < 0.25. The Polymorphism Information Content (PIC) is a very important measure of DNA polymorphism, genetic variation and gene mapping. In addition, the PIC is the measure of marker informativeness, ranging from zero to one. According to this classification, the PIC value of a locus close to 1.0 with many alleles is the desirable marker for genetic diversity evaluations and analysis. Markers with PIC values lower than 0.5 are moderate informative tools, whereas those with PIC ≥ 0.5 are highly informative to detect unique loci/alleles.

The effective number of alleles (N_e) is a reciprocal of gene homozygosity [64] and, as a result, the effective number of alleles can be used to predict the H_e. When heterozygosity is high, N_e is also high. The N_e of RAPD primers ranged from 1.27 with OP-B18 to 1.73 with RMN-P3, with an average of 1.39 over all primers, while it varied from 1.0 with UBC-810 to 1.59 with UBC-812 and UBC-814, with an average of 1.36 over all ISSR primers.

The difference in the rate of variation among different marker systems may be related to the differences in the source of the detected diversity. Each molecular marker analysis targets a different locus or different loci in the genome. The evaluation and characterization of accessions at the molecular level help in the identification of genetic variations present in the germplasm that can be used to improve the desired traits. Morphological (phenotypic) characters are usually affected by environmental conditions [65]; therefore, their use for classification would be misleading. The use of molecular markers has become a common practice in studies of population structure, genetic diversity for pre-breeding, marker-assisted crop breeding and in distinguishing proprietary genotypes for breeding and intellectual property rights [66].

Based on the RAPD analysis, the highest pair similarity was recorded for Samnut-26 and NC-7 (0.89), followed Samnut-22 and Samnut-26 (0.86). The lowest pair similarity was recorded for Ismailia-1 and Ex-Dakar (0.62) (Table 7a). The similarity matrices based upon ISSR showed a maximum pair

similarity of 0.97 for Samnut-22 and Samnut-26, followed by Samnut-26 and NC-7 (0.94). The lowest pair similarity of 0.60 was recorded for Ismailia-1 and NC-7 (Table 7b). Based on both RAPD and ISSR analyses (Table 7c), the highest pair similarity of 0.92 was recorded for Samnut-26 and NC-7 and for Samnut-22 and Samnut-26. The lowest pair similarity of 0.63 was recorded for Ismailia-1 and NC-7.

Table 7. Similarity matrices among peanut cultivars according to Jaccard Coefficient based on RAPD and ISSR analyses.

Cultivars	Ismailia-1	Ex-Dakar	Samnut-22	Samnut-26	NC-7
(a) Based on RAPPD analysis					
Ismailia-1	1.00				
Ex-Dakar	0.62	1.00			
Samnut-22	0.76	0.86	1.00		
Samnut-26	0.68	0.85	0.86	1.00	
NC-7	0.66	0.82	0.83	0.89	1.00
(b) Based on ISSR analysis					
Ismailia-1	1.00				
Ex-Dakar	0.67	1.00			
Samnut-22	0.67	0.83	1.00		
Samnut-26	0.65	0.86	0.97	1.00	
NC-7	0.60	0.86	0.91	0.94	1.00
(c) Based on RAPD and ISSR analyses					
Ismailia-1	1.00				
Ex-Dakar	0.65	1.00			
Samnut-22	0.71	0.85	1.00		
Samnut-26	0.66	0.86	0.92	1.00	
NC-7	0.63	0.84	0.88	0.92	1.00

Figure 1 shows the clustering of cultivars from the phenogram generated by UPGMA cluster analysis. The RAPD analysis formed two groups at a distance of 0.678. The first one includes Ismailia-1 only. The second group is further divided into two sub-groups at a distance of 0.842. The first sub-group includes Samnut-22 and Ex- Dakar cultivars at a distance of 0.862, while the second sub-group consists of NC-7 and Samnut-26 cultivars at a distance of 0.889. ISSR analysis revealed two groups at a distance of 0.645.

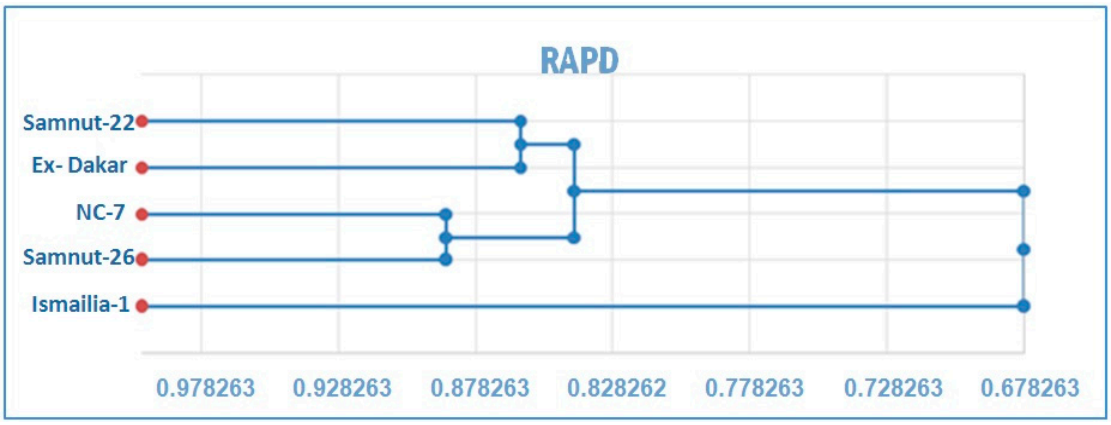


Figure 1. *Cont.*

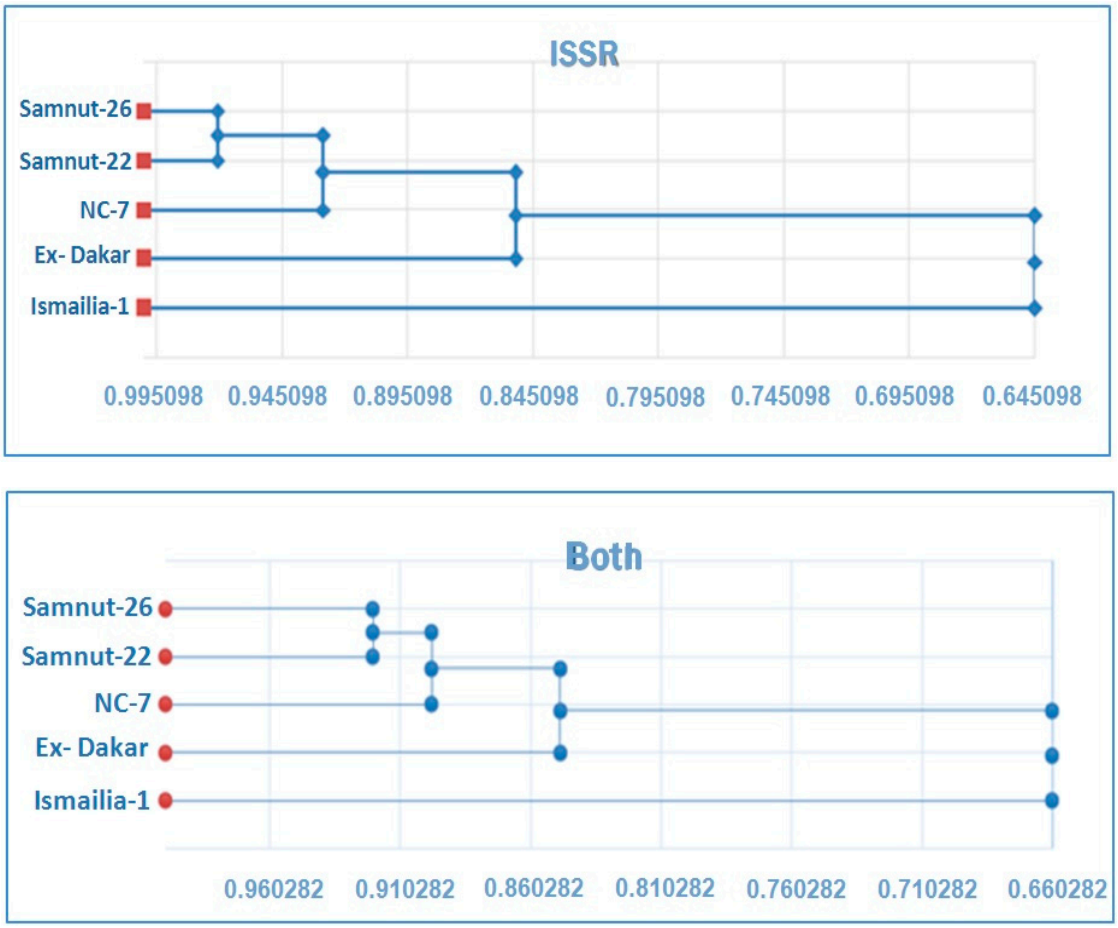


Figure 1. Phenogram generated by unweighed pair group method arithmetic average (UPGMA) cluster analysis, based on Nei and Li Coefficient, showing the cluster of peanut cultivars.

The first group consists of Ismailia-1 only, while the second group is further divided into two subgroups at a distance of 0.852. The first subgroup includes Ex-Dakar, while the other subgroup is further split into two sub-subgroups at a distance of 0.929. The first one includes NC-7 only, whereas the other one includes Samnut-22 and Samnut-26 at a distance of 0.971. The combined molecular analyses split the cultivars into two groups at a distance of 0.66. The first group consists of Ismailia-1 only, while the other group is further split into two subgroups at 0.849 with Ex-Dakar itself, while the other subgroup is further divided into two sub-subgroups at a distance of 0.898. The first one consists of NC-7 only and the second right sub-subgroup includes Samnut-26 and Samnut-22 at a distance of 0.921. In fact, the ISSRs have a high capacity to reveal polymorphisms and offer great potential to determine intra and inter genomic diversity compared to other arbitrary primers like RAPDs [67]. Studies on *Jatropha curcas* [68] and *Lactuca sativa* L. [9] indicated a similar difference between RAPDs and ISSRs' resolution. The two-marker techniques target different portions of the genome. The ability to resolve genetic variation among different genotype maybe more directly related to the number of polymorphisms detected with each marker technique rather than a function of which technique is employed. The differences found among the dendrogram generated by RAPDs and ISSRs could be partially explained by the different number of PCR products analyzed (735 for RAPD and 646 for ISSR), again reinforcing the importance of the number of loci and their coverage of the overall genome. Loarce et al. [69] observed the importance of the number of markers in determining the genetic relationships between rye genotypes. Another explanation could be the low reproducibility of RAPDs [70]. In this respect, Osman and Ali [71] indicated that ISSR and SCoT markers are considered powerful markers for the discrimination and identification of five studied

Lathyrus species. Moreover, in a study by Akçal-Giachino [72], the RAPD and ISSR marker systems were found to be useful for determining genetic diversity in *P. anisum* L. and identifying variations among landraces. The differences in the clustering patterns of genotypes using RAPD and ISSR markers may be attributed to marker sampling errors and/or the level of polymorphism detected, again reinforcing the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars [69].

3.4. Size and Number of Oil Bodies

The morphological profiles of oil bodies of all cultivars exhibited spherical or ovoid shapes, except Samnut-22, where the oil bodies were rectangular in shape. Generally, there was a negative relationship between size (diameter) and the number of oil bodies in all tested cultivars (R^2 = 0.82). Ex-Dakar and Samnut-22 showed a high number of oil bodies compared to other cultivars, with median diameters (D50) of 2.7 and 2.9 µm, respectively (Table 8 and Figure 2). Samnut-26 and NC-7 cultivars showed a narrower distribution, with about 50% of the oil bodies compared to other cultivars, but bigger in size. Ismailia-1 contained large oil bodies with D50 of 6.7 µm. The number of existing oil bodies in a slide measurement indicated the difference in abundance among cultivars (17, 59 and 63% in Ismailia-1, Samnut-26 and NC-7, respectively). Samnut-22 and Ex-Dakar had a higher abundance (82 and 80%, respectively) (Table 8).

Table 8. Mean diameter and number of oil bodies in seed cells of different peanut cultivars.

Cultivars	Oil bodies	
	Diameter (µm)	Number
Ismailia-1	6.80a †	17c
Ex-Dakar	2.95d	82a
Samnut-22	2.77d	80a
Samnut-26	4.27b	59b
NC-7	3.30c	63b

† Values followed by the same letters within a column for each cultivar are not significantly different (p = 0.05) based on Fisher's LSD test.

The morphological characterization of peanut seeds revealed that Ismailia-1, NC-7, and Samnut-26 cultivars had bigger sizes. Ex-Dakar and Samnut-22 were smaller in size (Figure 2A). The mean diameters and numbers of oil bodies were significantly different among cultivars. Ismailia-1 oil bodies had the largest size and smallest number (6.80 µm in diameter and 17 in number), followed by Samnut-26 (4.27 µm in diameter and 59 in number), NC-7 (3.30 µm in diameter and 63 in number), Ex-Dakar (2.95 µm in diameter and 82 in number), and Samnut-22 (2.77 µm in diameter and 80 in number) (Table 8 and Figure 2B).

Oil bodies can be found in different sizes in the same seed, but in different cells [73]. It is likely that the size of the oil bodies is affected by environmental factors such as the nutritional status of the plants (e.g., available nitrogen for oleosins and phosphate for PL). Variations among tested cultivars in terms of oil body size and number were documented in this study. In terms of oil body size, cultivars can be ranked as follows: Ismailia-1 > Samnut-26 > NC-7 > Ex-Dakar ≥ Samnut-22. This variation can be explained by the different amounts of acidic amino acid and protein structure on the surface of the oil bodies among cultivars [74]. For example, Ismailia-1 and Samnut-26 showed a wider diameter distribution than Ex-Dakar and Samnut-22, because Ex-Dakar contained more protein, and NC-7 contained much more oil. More protein was attached to oil bodies in Ex-Dakar and Samnut-22. The distributions of protein bodies and oil bodies seemed to be negatively correlated. A high oil content could mean a low protein content, and vice versa. The peanut protein and oil body extraction method may affect the yield of peanut protein and oil bodies. A peanut protein yield of 78.60% and an oil body yield of 48.44% were achieved under the optimal condition [36]. Oil bodies accumulate more

in seeds' embryo than in the endosperm [37]. This revealed that the ultrastructure of the endosperm and embryo significantly changed with the accumulation process of the oil bodies and fatty acid composition during the development [38].

In conclusion, the cultivar effect was evident in relation to seed production, quality, the size and number of oil bodies, and oil production. The effect of season was not evident, likely due to the stability of climatic conditions during the two seasons of the study. Genetic variations among peanut cultivars is of great importance in genotype selection based on stable segregating markers and could be very helpful in the conservation of germplasm resources. The cultivar Ismailia-1 had the highest oil yield and content of oleic acid in the present study, and could be a better choice for use as a parent in future breeding efforts to enhance oleic acid and oil content in peanut. The size of the oil body has been reported on in relation to the high oil yield of the Ismailia-1 cultivar compared to the number of oil bodies in Ex-Dakar and Samnut-22 cultivars, which reported low oil yields.

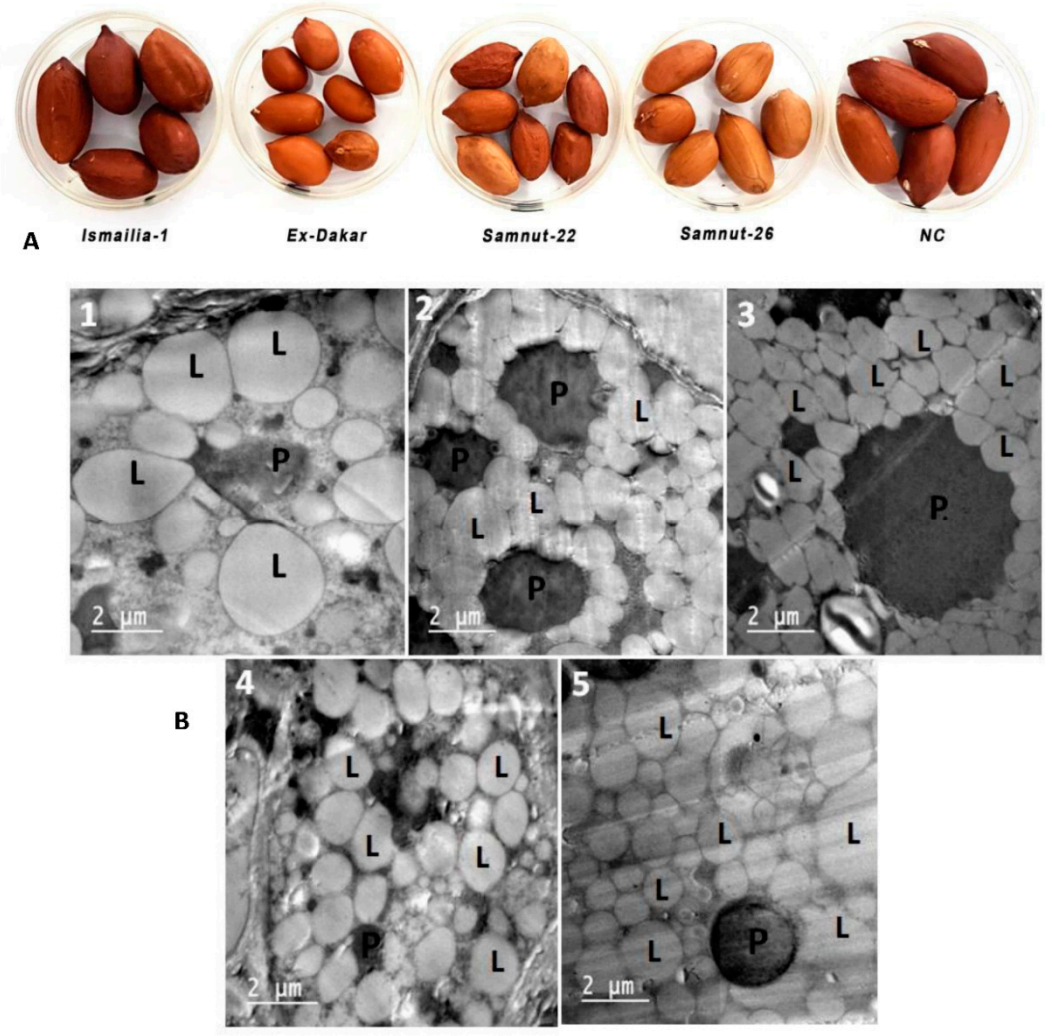


Figure 2. (**A**) Picture of five cultivar seeds. (**B**) Transmission electron microscope (TEM) picture of five peanut cultivars: (1) Ismailia-1, (2) Ex-Dakar, (3) Samnut-22, (4) Samnut-26, and (5) NC-7. Bars represent 2 µm (P—protein bodies; L—oil bodies).

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