281
Arab Univ.
J. Agric. Sci.,
Ain Shams Univ.,
Cairo, 26(1), 281-290, 2018

DEVELOPMENT OF RAPD AND SSR MARKERS ASSOCIATED WITH OIL CONTENT IN FIVE PEANUT CULTIVARS

[21]

Ghada, M. Samaha[1]; M.A. Rashed[2]; A.H. Atta[2] and M.A. Ahmed[1]
1- Field Crops Dept., National Research Center, Dokki, Giza, Egypt
2- Genetics Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt

Keywords: Peanut, Oil content, RAPD and SSRs markers

ABSTRACT

The peanut (*Arachis hypogaea* L.) is an important oilseed crop in tropical and subtropical regions of the world. Oil content has an important quality trait for peanut. However, the progress in genetic improvement of oil content is slow. Therefore, identification of molecular markers for oil content trait is a great impact in molecular breeding. Nineteen RAPD and ten SSR primers were used to detect markers related to oil content in peanut. The five peanut cultivars were grown for two seasons (2013, 2014) in El-Nubaria, Al-Beheira Governorate, and Egypt. The results showed that, Gregory cultivar recorded the highest value of oil content, while Giza5 cultivar exhibited the lowest value of oil content in both seasons. The results indicated the presence of five positive and nine negative RAPD markers and two positive and one negative SSR markers that could be considered as reliable markers for oil content in peanut**.**

INTRODUCTION

Peanut, also called groundnut (*A. hypogaea* L.), is a grain legume and oilseed, which is widely cultivated in tropical and subtropical regions (annual production of ~46 million tons). It has a key role in human nutrition (**FAO**, **2015**). China and India are the biggest peanut producers as they produce nearly 60% of the world's yield (**USDA**, **2010)**. All parts of the peanut plant can be utilized and it can be used in various types of industries.

Peanut seeds contain high amounts of carbohydrate (20%), protein (25-30%), edible oil (43 - 50%) and fiber (5%) and ash which make a substantial contribution to human nutrition, including unsaturated fatty acids, tocopherols, phytosterols and phenolic compounds and antioxidants such as oleic acid, while also being one of the most widely used legumes due to their taste (**Akhtar et al 2014**).

The oleic to linoleic acid ratio is considered to be an indicator of peanut oil stability, and it is a shelf-life index for industrial applications (**Bolton & Sanders, 2002 and Campos-Mondragón et al 2009**).

Over 60% of global peanut production is crushed for extraction of oil for edible and industrial uses, while 40% is consumed in food uses and others such as seeds for sowing the next season crop (**Birthal et al 2010).**

Increasing the growing area of oil seed crops out of the Nile valley is considered as a good way to reduce the competition with other main summer crops (maize, rice and cotton). Peanut can be grown successfully in newly reclaimed sandy soil; this approach is needed in increasing seed and oil production **(Shaban et al 2009).** It can play an important part in decreasing the gap between production and consumption (~90%) in Egypt (**FAO, 2009**). The cultivated area of peanut in Egypt during 2014 was about 57, 321 hectares with a total production of 183,438 tons (**FAO**, **2015).**

Oil content has been an important quality trait for peanut. However, the progress in genetic improvement of oil content is slow. This is mainly due to the complex genetic basis, the high cost in oil content testing, and difficulty in phenotypic selection because of environmental influence. Therefore, marker assisted selection (MAS) is necessary for oil content improvement in peanut breeding **(Huang et al 2011)**.

(Received 15 June, 2017)
(Revised 9 July, 2017)
(Accepted 10 July, 2017)

Random amplified polymorphic DNA (RAPD) became one of the widespread DNA techniques, a quick method for developing genetic maps and to determine DNA fragments to discriminate peanut cultivars (**Azzam & El-Sawy, 2005 and Guo et al 2005).** It offers the simplest and fastest method for detecting a great number of genomic markers in less period of time (**Edwards et al 1992**).

The molecular markers are efficiently employed in breeding programs MAS. Markers not only eliminate the need of chemical analysis and phenotypic evaluation in the early generation breeding program, but also minimize the time required to develop new genotypes with desirable traits. Among the molecular markers, Simple Sequence Repeats (SSRs) are useful for marker-assisted selection of oil content in peanut (**Eskandari et al 2013**).

SSRs are codominant marker thus heterozygote produces two bands revealing the amplification of the two loci and could be readily identified. SSR co-dominancy increases the accuracy and efficiency of population genetic measures based on these markers compared with other markers (**Wu et al 2010**).

The objective of this study was to detect DNA markers associated with oil content in peanut using RAPD and SSR-PCR techniques.

MATERIALS AND METHODS

Materials

Five peanut cultivars namely; Giza 5, Giza 6, Ismailia-1, Gregory and R92 were used in this study, they were obtained from Oil Crop Research Department, Field Crops Institute, Agriculture Research Center, Giza, Egypt. This study were conducted at the Department of Genetics, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt and Field Crops Department of the National Research Center, Dokki, Giza, Egypt during the period from 2012 to 2017.

Methods

Field experiment

These five peanut cultivars were grown for two seasons (2013, 2014) in a randomized complete block design with five replications at the Research and Production Station of the National Research Center in El-Nubaria, Al-Beheira Governorate, Egypt, to evaluate the performance of these cultivars for oil content and some related traits. The data were recorded for oil, Oleic acid, Linoleic acid contents and O/L ratio traits in both the two seasons. Oil content was estimated using Soxhlet apparatus, while oil quality with respect to Oleic acid and Linoeic acid contents was estimated using Gas Chromatography according to **A.O.C.S. (2012)** O/L ratio was calculated as the ratio of Oleic acid to Linoleic acid.

Statistical analysis

The collected data for these four traits from the five tested peanut cultivars were statistically analyzed using analysis of variance (ANOVA) procedure according to **Snedecor and Cochran (1969)**. The differences among means were compared using Duncan's new multiple ranges test (**Duncan, 1955).**

Molecular genetic studies

Genomic DNA extraction

Total genomic DNA was extracted from dry seeds of the five tested peanut cultivars according to **(Yu et al 2010)**. Approximately a 0.2g of dried seed was placed into a 1.5 ml tube with a 200µl of DNA extraction buffer [10 mM Tris-HCl (pH 7.6), 5 mM EDTA, 0.5% SDS, 0.5% NP-40, 0.5% Tween-20, 5 mg/ml PVP 40, 80 µg/ml proteinase K] and ground with a plastic pestle until a milky-white solution or a paste was formed. The tube was then incubated in a 55ºC water bath for 20 min for cell lysis and protein digestion. After digestion was completed, a 200µl of phenol-chloroform-isoamylol (25:24:1, V/V/V) was added to the tube to remove proteinase K. After centrifugation at 9,000 xg for 5 min, the supernatant was collected (~150 µl) in a sterile eppendorf tube with an equal volume of isopropanol. The mixture was gently mixed and centrifuged at 10,000 xg for 2 min to precipitate the DNA. The dried DNA pellets were then dissolved in a 150 µl of TE buffer.

RAPD-PCR analysis

PCR reactions were performed according to (**Williams et al 1990)** using 19 arbitrary 10-mer primers (**Table 1**). The reaction conditions were optimized and mixtures (25 µl total volume) consisted of a 2 µl of DNA template (25 ng / µl), a 2 µl of primer, a 0.5 µl of dNTPs (2.5 mM), a 2.5 µl of Mgcl2 (2.5 mM), a 2.5 µl of 10X buffer, Taq DNA polymerase (1U/ µl) and H_2O up to 25 µl. Amplifi-

cation was carried out in a Primus Thermocycler, which programmed for 37 cycles as follows; denaturation, 94°C/2 min (one cycle), annealing, 94°C / 1 min, 37°C/1 min, 72°C/2 min (35 cycles), extension, 72°C/10 min (one cycle), then kept at 4°C until use. Agarose gel (1.5 %) electrophoresis was used for separating the PCR products. The run was performed at 100 volts for about one hour. DNA marker used in this study was 1kb DNA ladder which consists of ten different DNA fragments (1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp)**.**

Table 1. List of the used 19 primers and their nucleotide sequences for RAPD-PCR analysis

Primer (OP-)	Sequence (5'→3')	Primer (OP-)	Sequence (5'→3')
A02	TGCCGAGCTG	B04	GGACTGGAGT
A04	AATCGGGCTG	B06	TGCTCTGCCC
A05	AGGGGTCTTG	B 10	CTGCTGGGAC
A07	GAAACGGGTC	D10	GGTCTACACC
A10	GTGATCGCAG	O14	AGCATGGCTC
A11	CAATCGCCGT	O15	TGGCGTCCTT
A16	AGCCAGCGAA	Z02	CCTACGGGGA
A17	GACCGCTTGT	Z12	TCAACGGGAC
A19	CAAACGTCGG	Z15	CAGGGCTTTC
B03	CATCCCCCTG		

SSR-PCR analysis

Ten developed SSR primers were used for the amplification of SSR bands among the five tested peanut cultivars as shown in **Table** (**2**) which were employed to detect DNA markers associated with oil content. These primers were selected from various published sources (**Moretzsohn et al 2005**; **Selvaraj et al 2009 and Anitha et al 2014**). Each 25-µL of PCR mixture for the amplification of SSR bands consisted of 12.6 µl (2X) of KAPA2G Fast Ready Mix[2], a 1µl of forward primer, a 1µl of reverse primer, a 2µl of DNA template and H_2O up to 25 µl. Amplification was carried out in a Primus Thermocycler, programmed for 37 cycles as follows; denaturation, 94°C/4 min (one cycle), annealing, 94°C / 1 min, 53–63°C /45 sec, 72°C/ 1 min (35 cycles), extension, 72°C/10 min (one cycle), then kept at 4°C until use. Agarose gel (3%) electrophoresis was used for separating the PCR products. The run was performed at 100 volts for about one hour. DNA Marker used in this study was 1 kb DNA ladder**.**

Analysis of gel images

All fragments resulting from RAPD and SSR gels were detected on an UV transilluminator filter. All gels were photographed under UV light with Polaroid film 667 and scanned with Bio- Rad video densitometer Model 620 at a wavelength of 577. The gel image was analyzed using the Total lab TL 120 to determine the molecular sizes of the amplified fragments. The amplified fragments were scored as present (1) or absent (0).

Table 2. The ten SSR primer-pairs names, their forward and reverse sequences and their required annealing temperature used with peanut cultivars.

Primers	Forward Sequences(5'→3')	Reverse Sequences(5'→3')	Ann. temp
PM 36	ACT CGC CAT AGC CAA CAA AC	CAT TCC CAC AAC TCC CAC AT	**53**
IPAHM 103	GCA TTC ACC ACC ATA GTC CA	TCC TCT GAC TTT CCT CCA TCA	**60**
pPGSseq 19D9	TGT TGC CCA CTG GTT CTA ATC A	TCA AAT GGC ATA GTC TCC CC	**63**
PM 204	TGG GCC TAA ACC CAA CCT AT	CCA CAA ACA GTG CAG CAA TC	**53**
PPGPseq2A5	GGG AAT AGC GAG ATA CAT GTC AG	CAG GAG AGA AGG ATT GTG CC	**63**
Ah2TeqE08	GAA ACA GCC GCG AGA GAA	CCC TAA CCT CTC TTC ATT GTG C	**55**
pPGpseq3B8	GGA GAA AGA TCA AAC GAG AAC A	TTC GAA TAT CTG ATT TGC TTT T	**63**
PPGSseq10H1A	TGA CAA TGG GGT GTT CTT CA	GTA AAC AGA CGC CGT TCC AT	**63**
pPGpseq2E6	TAC AGC ATT CCC TTC TGG TG	CCT GGG CTG GGG TAT TAT TT	**63**
pPGPseq7H6	CAT CCT CAC GGG AGT CAG AT	ATA CCT ACG CGT TGT GGA GC	**63**

Ann.temp. = annealing temperature

RESULTS AND DISCUSSION

Field experiment data analysis

Analysis of variance for oil, Oleic acid, Linoleic acid contents and O/L ratio traits revealed significant variations among the five tested peanut cultivars and summarized in the **Table** (**3**). Means of oil content trait of all cultivars in 2013 season were higher than in 2014 season. These differences may be due to difference in the genetic make up of the tested cultivars and also oil content trait is often influenced by environment. Mean of oil content trait values were significantly varied among the tested cultivars, which ranged from 53.3 and 52.8% for Gregory to 45.2 and 45.1% for Giza-5 in the 2013 and 2014 seasons, respectively. It was clear that the highest value of Oleic acid content (57.7, 52.8%) and O/L ratio (2.6, 2.1) traits were recorded by Gregory cultivar in the two seasons. Although, this cultivar gave the lowest Linoleic acid content (22.6, 27.1%) in the two seasons. Gregory cultivar was more suitable for oil quality as compared with the other cultivars.

According to their oil content trait, Gregory cultivar was chosen as the highest oil content one, while Giza 5 cultivar was chosen as the lowest oil content one and the other 3 cultivars in the midst as shown in **Table** (**3**). The two cultivars; Gregory and Giza 5 were used to obtain RAPD and SSR markers associated with oil content.

These results are in harmony with those obtained by **(Migawer et al 2001 and El-Saady et al 2014)** who found significant differences among the tested cultivars concerning seed oil and protein contents. Similar results were obtained by **Mahrous et al (2015)** who found that Gregory cultivar showed the highest seed oil content (52.22 %). Also, **Gulluoglu et al (2016)** found that oil content of peanut varieties varied between 46.96-51.55% on based of dry weight and the highest oil content (51.55%) was reported in Georgia Green and the lowest (46.96%) was in Flower-22 varieties.

RAPD markers for oil content

DNA isolated from the five peanut cultivars were tested against nineteen primers. The nineteen primers revealed a polymorphism with the five tested peanut cultivars, which only ten developed molecular markers for oil content as shown in **Figure (1)** and summarized in **Table (4).**

OP-A05, OP-A07, OP-A10, OP-D10 and OP-Z12 primers exhibited five positive molecular markers with molecular sizes of 241 bp for OP-A05, 489 bp for OP-A07, 443 bp for OP-A10, 451 bp for OP-D10 and 413 bp for OP-Z12 primers, which were found only in the highest oil content cultivar (Gregory), while they were absent in the all other cultivars beside the lowest oil content one (Giza 5).

On the other hand, OP-A04, OP-A07, OP-A11, OP-B04, OP-Z02 and OP-Z15 primers exhibited nine negative molecular markers with molecular sizes of 811 bp for OP-A04, 224bp for OP-A07, 584 bp for OP-A11, 801, 680, 172 and 141bp for OP-B04, 377 bp for OP-Z02 and1104 bp for OP-Z15 primers, which were found only in the lowest oil content cultivar (Giza 5), while they were absent in the all other cultivars beside the highest oil content one (Gregory).

Table 3. Means of oil, oleic acid, linoleic acid contents and O/L ratio traits for the peanut cultivars at 2013 and 2014 seasons.

season	Season 2013				Season 2014			
Trait / Cultivars	Oil content	Oleic acid	Linoleic acid	O/L ratio	Oil content	Oleic acid	Linoleic acid	O/L ratio
Giza 5	45.2^{c}	42.2^{cd}	30.4^{ab}	1.4 ^{b}	45.1^{c}	42.6^{abc}	30.4^{ab}	1.4 ^{b}
Giza 6	49.1^{b}	47.0^{b}	32.2^{a}	1.5 ^{b}	49.0^{b}	46.3^{ab}	32.9^{a}	1.4 ^{b}
Gregory	53.3^{a}	57.7^{a}	22.6^{c}	2.6 ^{a}	52.8^{a}	56.3^{a}	27.1^{c}	2.1 ^{a}
Ismailia	48.8^{b}	44.3^{c}	27.6^{b}	1.6 ^{b}	48.7^{b}	45.1^{ab}	30.4^{ab}	1.5 ^{b}
R92	48.9^{b}	40.2^{d}	27.8^{b}	1.4 ^{b}	48. 5^{b}	41.1^{c}	30.1^{ab}	1.4 ^{b}

Means with the same letter(s) in the column are not significantly different by Duncan's new multiple range test (P <0.05).

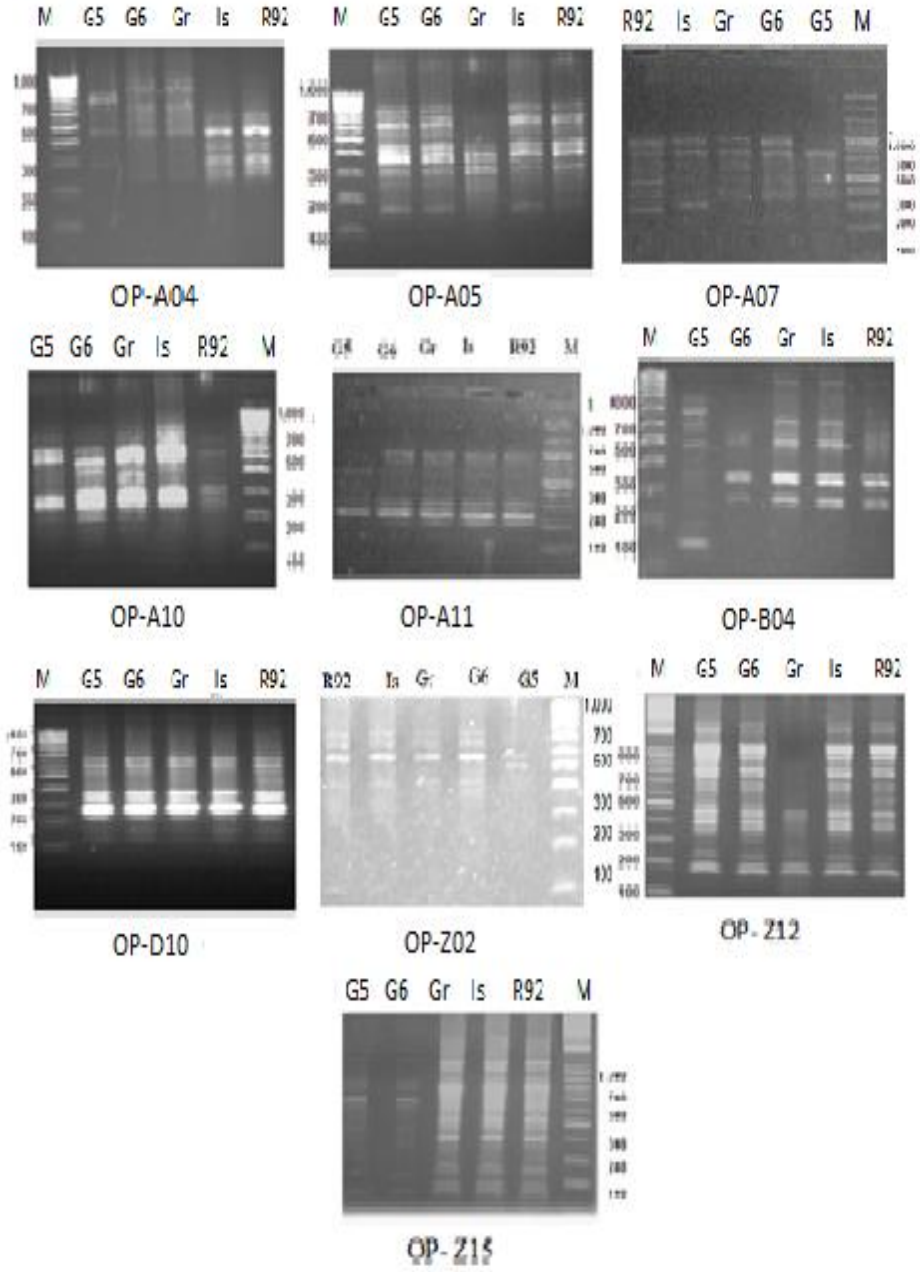

Fig. 1. RAPD-PCR fragments of the ten primers (OP-A04, OP-A05, OP-A07, OP-A10, OP-A11, OP-B04, OP-D10, OP-Z02, OP-Z12 and OP-Z15) with the five tested peanut cultivars (M= Marker, G5 = Giza 5 as a lowest oil content , G6 = Giza 6, Gr = Gregory as a highest oil content , Is = Ismailia-1 and R92).

Table 4. Presence and absence of RAPD-PCR markers of the ten tested primers with the five tested peanut cultivars (G5 = Giza 5 as a lowest oil content, G6 = Giza 6, Gr = Gregory as a highest oil content, Is = Ismailia 1 and R92).

Primer No.	Primer name	TAF	Band No.	MS (bp)	G5	G6	Gr	IS	R92	Polymorphism %	MT
1	**OP-A04**	9	2	811	1	0	0	0	0	77.78	N
2	**OP-A05**	9	8	241	0	0	1	0	0	55.56	P
3	**OP-A07**	8	3	489	0	0	1	0	0	75.00	P
			7	224	1	0	0	0	0	60.00	N
4	**OP-A10**	10	6	443	0	0	1	0	0	60.00	P
5	**OP-A11**	5	3	584	1	0	0	0	0	81.82	N
6	**OP-B04**	11	2	801	1	0	0	0	0		N
			3	680	1	0	0	0	0		N
			10	172	1	0	0	0	0		N
			11	141	1	0	0	0	0		N
7	**OP-D10**	8	4	451	0	0	1	0	0	37.50	P
8	**OP-Z02**	6	4	377	1	0	0	0	0	83.33	N
9	**OP-Z12**	17	11	413	0	0	1	0	0	52.94	P
10	**OP-Z15**	13	3	1104	1	0	0	0	0	61.54	N
	Total		14		9	0	5	0	0		

TAF= total amplified fragments MS=molecular size MT=marker type P=positive N=negative

These five positive and nine negative RAPD markers could be considered as reliable markers or oil content in these peanut cultivars.

These results agreed with (**Rajcan et al 1999**) reported a RAPD markers associated with seed oil content in *B. napus***.**

SSR markers for oil content

SSR primers were used in this study to detect DNA markers related to oil content. DNA isolated from the five peanut cultivars were tested against four pre-selected primers as shown in **Figure (2)** and summarized in **Table (5)**. Out of the ten used primers, only four primers revealed a polymorphism with the five peanut tested cultivars, which only two primers developed molecular markers for oil content.

PM 36 primer exhibited two positive SSR markers with molecular sizes of 255 bp and 204 bp, which were found only in the highest oil content cultivar (Gregory), while they were absent in the all other cultivars beside the lowest oil content one (Giza 5).

IPAHM 103 primer exhibited only one negative SSR marker with molecular size of 209 bp, which was found only in the lowest oil content cultivar (Giza 5) and was absent in the all other cultivars beside the highest oil content one (Gregory).

These two positive and one negative SSR markers could be considered as reliable markers for oil content in these peanut cultivars. These results agreed with many reports detected SSR markers for oil content, **Selvaraj et al (2009)** who showed that PM36 SSR marker was associated with oil content in peanut. At the same time **Huang et al (2012)** found another SSR marker, 2A5-250/240, was tightly linked to the oil content trait in peanut cultivars. **Anitha et al (2014**) reported that the PM36, PGS19D09 and IPAHM103 primers were associated with oil content of groundnut. **Sheng et al (2016)** used SSRs marker for understanding the genetic control of seed oil content in *B. napus*. These results could contribute to the enhancement of oil yield through early prediction of high oil content peanut cultivars using RAPD and SSR markers.

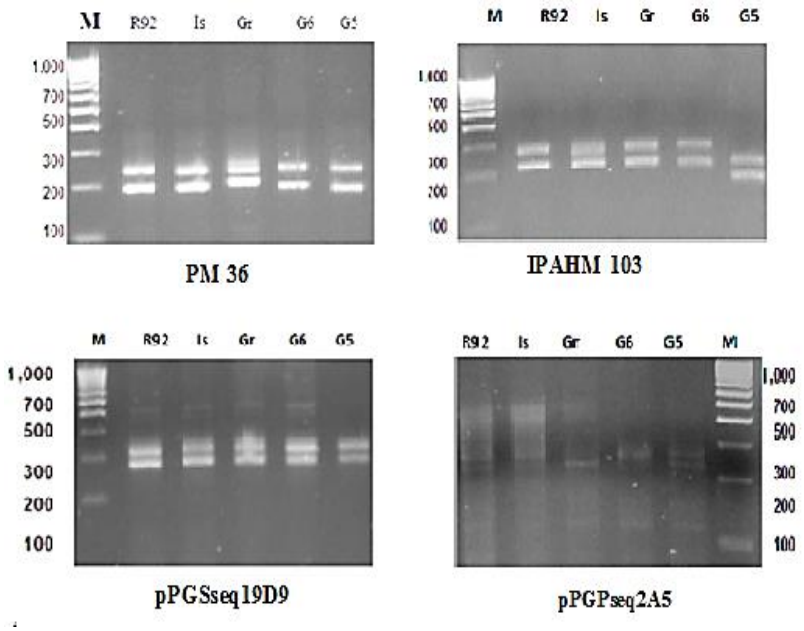

Fig. 2. SSR-PCR fragments of the four used primers (PM 36, IPAHM 103, pPGSseq19D9 andPGPseq2A5) with the five tested peanut cultivars (M= Marker, R92, Is=Ismailia-1, Gr = Gregory as a highest oil content, G6 = Giza 6, and G5 = Giza 5 as a lowest oil content).

Table 5. Presence and absence of SSR-PCR fragments of the four tested primers with the five tested peanut cultivars (R92 , Is=Ismailia-1, Gr = Gregory as a highest oil content, G6 = Giza 6, and G5 = Giza 5 as a lowest oil content).

Primer No.	Primer name	TAF	Band No.	MS (bp)	R92	IS	Gr	G6	G5	Polymorphism	MT	P %
1	**PM 36**	4	1	255	0	0	1	0	0	Unique	P	75
			2	239	1	1	1	1	1	Monomorphic	---	
			3	204	0	0	1	0	0	Unique	P	
			4	193	1	1	0	1	1	Polymorphic	---	
2	**IPAHM 103**	3	1	285	1	1	1	1	0	Polymorphic	---	66.67
			2	269	1	1	1	1	1	Monomorphic	---	
			3	209	0	0	0	0	1	Unique	N	
3	**pPGSseq19D9**	4	1	589	1	1	1	1	0	Polymorphic	---	50
			2	526	1	1	1	1	0	Polymorphic	---	
			3	353	1	1	1	1	1	Monomorphic	---	
			4	291	1	1	1	1	1	Monomorphic	---	
4	**pPGPseq2A5**	3	1	260	1	1	0	1	1	Polymorphic	---	33.33
			2	244	1	1	1	1	1	Monomorphic	---	
			3	114	1	1	1	1	1	Monomorphic	---	
	Total	14	14		11	11	11	11	9			

MS= molecular size MT= marker type P= positive N= negative P % = polymorphism percentage

CONCLUSION

The results of the field experiment showed that Gregory revealed the highest oil content cultivar, while Giza 5 cultivar showed the lowest oil content one. The present study used RAPD and SSR primers to detect some markers related to oil content. RAPD-PCR exhibited five positive and nine negative markers, while SSR exhibited two positive and one negative markers for high oil content. The information generated through this study indicated that there are potential markers for use in early prediction to improve oil content trait in peanut cultivars by molecular breeding program**.**

REFERENCES

Akhtar, S., Khalid, N., Ahmed, I., Shahzad, A. and Suleria, H.A.R. 2014. Physicochemical characteristics, functional properties, and nutritional benefits of peanut oil: **J. Critical Reviews in Food Sci. and Nutrition. 54 (12), 1562-1575.**

Anitha, B.K., Manivannan, N. and Anandakumar, C.R. 2014. Validation of SSR markers associated with QTLs for pod and kernel traits in groundnut (*Arachis hypogaea* L.): International **J. of Agri. Sci., 4(1), 077-082.**

A.O.C.S, 2012. In Official Methods and Recommended Practices of the American Oil Chemists' Society. 4th ed, Champaign. IL, USA.

Azzam, Clara R. and El-Sawy, W. 2005. Variation induced via gamma radiation in peanut (*Arachis hypogaea* L.) and its assessment by protein finger printing. **Egypt J. Plant Breed, 9 (2), 237-256.**

Bolton, G.E. and Sanders, T.H. 2002. Effect of roasting oil composition on the stability of roasted high-oleic peanuts. **J. of the American Oil Chemists' Society, 79, 129–132.**

Birthal, P.S., Rao, P.P., Nigam, S.N., Bantilan, C. S. and Bhagavatulu, S. 2010. Groundnut and Soybean Economies in Asia: Facts, Trends and Outlook. Patancheru: **International Crops Research Institute for the Semi-Arid Tropics, 92 p.**

Campos-Mondragón, M.G., Calderón de la Barca, A.M., Durán-Prado, A., Campos-Reyes, L.C., Oliart-Ros, R.M., Ortega-García, J., Angulo, O. 2009. Nutritional composition of new peanut (*Arachis hypogaea*) cultivars. **Grasasy Aceites, 60, 161–167.**

Duncan, D.B. 1955. Multiple ranges and multiple F test. In: **Biometrics, 11, 1-42.**

Edwards, M.D, Helntijaris, T.T., Wright, S. and Stuber, C.W. 1992. Molecular marker facilitated investigation of quantitative traitLoci in maize. 4. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism and restriction fragment length polymorphism, **Genome, 43(3), 449-451.**

El-Saady, A.M., El-Sayed, A.A., Tellep, W.M.A.K. and El-Dahshour, M.F. 2014. Response of some peanut (*Arachis hypogaea* L.) cultivars grown in sandy soil to soil and foliar feeding with the different sources of phosphorus. **Inter. J. of Plant and Soil Sci., 3(6), 523-537**

Eskandari, M., Cober, E.R. and Rajcan, I. 2013. Genetic control of soybean seed oil: I. QTL and genes associated with seed oil concentration in RIL populations derived from crossing moderately high-oil parents. **Theor Appl Genet., 126, 483-495**

FAO, 2009. Food and Agriculture Organization of the United Nations. Global Market Analysis, **178 p.,** Rome, Italy.

FAO, 2015. Food and Agriculture Organization of the United Nations. Global Market Analysis, **76 p.,** Rome, Italy.

Gulluoglu, L., Bakal, H., Onat, B., EL Sabagh, A. and Arioglu, H. 2016. Characterization of peanut (*Arachis hypogaea* L.) seed oil and fatty acids composition under different growing season under mediterranean environment. **J. of Experimental Biology and Agric. Sci., 4, 2320.**

Guo, B., Holbrook, C., He, G., Culbreath, A. and Kvien, C. 2005. Identification and characterization of molecular marker(s) associated with resistance to TSWV and leaf spots in peanuts. **Planta, 223, 512-520.**

Huang, L., Chen, Y., Ren, X., Tang, M. and Zhou, X. 2012. Genetic diversity of *Arachis* accessions by EST-SSR from cultivated peanut (*Arachis hypogaea* L.). **Acta Agron Sin, 38, 1221–1231.**

Huang, L., Zhao, X., Zhang, W., Fan, Z. and Ren, X. 2011. Identification of SSR markers linked to oil content in peanut (*Arachis hypogaea* L.) through RIL population and natural population. **Acta Agron Sin 37, 1967–1974.**

Mahrous, N.M., Safina, S.A., Abo Taleb, H.H. and El-Behlak, S.M.E. 2015. Integrated use of organic, inorganic and bio fertilizers on yield and quality of two peanut (*Arachis hypogaea* L.) cultivars grown in a sandy saline soil. **American-Eurasian J. Agric. & Environ. Sci., 15(6), 1067-1074.**

Migawer, E.A. and Soliman, A.M. 2001. Performance of two peanut cultivars and their response to NPK fertilization in newly reclaimed loamy sandy soil. **J. Agric. Sci. Mansoura Univ., 26(11), 6653-6667**.

Moretzsohn, M.C., Leoi, L., Proite, K., Guimara, P.M., Leal-Bertioli, S.C.M., Gimenes, M.A., Martins, W.S., Valls, J.F.M., Grattapaglia, D. and Bertioli, D.A.J. 2005. Microsatellite-based gene-rich linkage map for the AA genome of *Arachis* (Fabaceae). **Theor. Appl. Genet., 111, 1060-1071.**

Rajcan, I., Kasha, K., Kott, L. and Beversdorf, W.D. 1999. Detection of molecular markers associated with linolenic and erucic acid levels in spring rapeseed (*Brassica napur* L.). **Euphytica, 105, 173 -181.**

Selvaraj, M.G., Narayana, M., Schubert, A.M., Ayers, J.L., Baring, M.R. and Burow M.D. 2009. Identification of QTLs for pod and kernel traits in cultivated peanut by bulked segregant analysis. **Electron. J. Biotech. 12, 1-10.**

Shaban, S.A., Abu-Hagaza, N.M., Mahrous, N.M. and El-Hameed, M.H.A. 2009. Effect of planting dates, hill-spacings and harvest dates on yield and quality of the new released peanut cultivar Giza 6. **Egypt. J. of Agron., 31(1), 39-54.**

Sheng, L., Chuchuan, F. Jiana, L., Guangqin, C., Qingyong, Y., Jian, W., Xinqi, Y., Chunyu, Z. and Yongming, Z. 2016. A genome-wide association study reveals novel elite allelic variations in seed oil content of *Brassica napus.* **Theoretical and Applied Genetics, 129(6), 1203–1215.**

Snedecor, C.W. and Cochran, W.G. 1969. In statistical methods 6th Ed. Iowa State Univ. Press, Ames, Iowa, USA.

USDA, 2010. United States Department of Agriculture. Foreign Agriculture Service (FAS), Peanut Area, Yield and Production, Washington, D.C., USA.

Williams, J.G.K., Kubelk, A.R., Livak, K.J. Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. **Nucl. Acid Res., 18, 6231-6235.**

Wu, N., Matand K., Wu, H., Li, B., Li, Y. and Zhang, X. 2010. *De novo* next-generation sequencing, assembling and annotation of *Arachis hypogaea* L. spanish botanical type whole plant transcriptome. **Theo rAppl Genet; 126, 1145–1149.**

Yu, S.T., Wang, C.T., Yu, S.L., Wang, X.Z., Tang, Y.Y., Chen, D.X. and Zhang, J.C. 2010. Simple method to prepare DNA templates from a slice of peanut cotyledonary tissue for Polymerase Chain Reaction. **Electronic J. of Biotechnology, 1(6), 077-082.**