THE EFFECT OF GENE POLYMORPHISM ON THE PRODUCTION AND QUALITY OF OIL IN THE SESAME PLANT SEED

Ghada Saad[1]*, Akeel H. ALassi[1] and Akeel N. AL mouhammedi[2]

[1]*Biology Department, College of Science, Tikrit University, Iraq
[2]Department of Field Crops, College of Agriculture, Tikrit University Iraq

Abstract

The study aimed to determine the level of gene expression of two genes (*SiPPO* and *SiNST1*) responsible for the production and quality of oil in sesame plant, using real-time PCR technique. In this study, used four varieties of sesame plant, Mahale, Wadaa, Rafideen and Somer. Sesame oil was extracted using Soxhlet extractor and then the percentage of oil for each variety was estimated, the highest percentage of oil was in the Mahale variety, as well as the percentage of five fatty acids are saturated and unsaturated: Palmatic Acid, Stearic Acid, Oleic Acid, lenolic Acid and Lenolinic Acid were measured by gas chromatography technique, while the Sesame lignans (antioxidants): sesamoline, sesamine and sesamol for studied species were measured by HPLC technique, the highest concentration of Sesame lignan was in Rafideen variety.

Key words: gene polymorphism, oil, environmental, plant seed.

Introduction

Seseamum indicum L. is known as ancient oil crop belonging to the Pedaliaceae family. Sesame seeds contains 44-60% oil, 18-25% protein, 10.5-13.5% ash (Abdalbasit *et al*., 2014). China, India and Sudan are the largest countries that produce sesame in the world (Cheung *et al*., 2007). About 70% of the sesame seeds are used for eating and oil extraction industries due to the high protein and lipid content and its distinctive flavor (Abuo-Gharbia *et al*., 2000), sesame oil is used in food and therapeutic purposes and sesame oil has been used in wounds treatment and as antibacterial against agents of dermal diseases such as streptococcus and staphylococcus, As well as used as antifungal and antiviral. In the Netherlands, the physicians has been used a sesame oil to treat a different of diseases such as hepatitis, migraines and diabetes also it's maintain a cholesterol level (HDL) in the blood (Sirato-Yasumoto *et al*., 2001). It's useful and easy to digest and does not freeze and doesn't lead any complications in the arteries and heart. Sesame oil is rich with unsaturated fatty acids, which plays a beneficial role for health especially heart health (Dachtler *et al*., 2003). Sesame seed rich in lignans compounds such as Sesamin, sesamolin and asarinin, have shown antioxidant, this compounds play an important role to effect on human health (Schwertner and Rios., 2012), such as antihypertensive (Nakano *et al*., 2008), neuroprotective activities (Cheng *et al*., 2008) and increasing hepatic fatty acid oxidation enzyme (Ashakumary *et al*., 1999). Enter into cooked candy and bread (Ajay *et al*., 2010) and used in manufacture of tippy and medical products and soaps (Nzikou *et al*., 2009). Sesame residues are used as feed for poultry and livestock because it's richness source of protein (Monteiro *et al*., 2014). A detection and estimation of gene expression (gene expression analysis) by real time polymerase chain reaction (PCR) is one of the most modern technics that recently used for many purpose such as early detection for diseases, determining a numbers of production genes in plant and distinguishing between species according the plant products (Robert, 2008). Gene expression analysis is provides an insight into complex biological processes and has become an integral part for many molecular biological studies (Bustin *et al*., 2005). Gas chromatography was an indispensable analytical technique in analysis of fatty acids in oil seeds. Also, it's used in the study of variance of fatty acids in oil crops (Abeer *et al*., 2017).

In study, oil was extracted and were RNA isolated from young leaves for studied species and worked all the

required experiments and recorded the results according to the steps for which they were prepared:

1. Measurement of oil percentage by GC in the studied species.

2. Measurement the concentration of active substances by HPLC in the studied species.

3. Use real time PCR technique to measure a level of gene expression for two association gene with oil production in sesame plant.

4. Finding relationship between a gene expression of the genes which responsible for oil production and quantity in the studied species and comparison among the four species in terms of oil production and quantity.

Materials and Methods

Samples preparation: The four species of sesame plant (Mahale, Somer, Rafideen, Wadaa) were cultivated in April 2019 and all samples taken after 45 day for study.

RNA gene isolation: RNA was isolated from fresh leaves four species using Quick-RNA plant miniprep kit that supplied from ZYMO research USA company, fresh leaves were collected and grinded using mortar, the samples were placed in mortar and liquid nitrogen added to crushed the cells. Then placed in ZR Bashing Bead Lysis tube after crushed and added 800μL RNA lysis buffer mixed for 30 second by vortex, then the tubes placed in centrifuge for 1 minute and pulled 400μl from top layer and placed in ZYMO-spin IIICG columns and centrifuged for 30s. then added 95-100% ethanol and well mixed. The mixture moved to ZYMO-spin IIC columns and centrifuged for 30s., then added RNA prep to ZYMO-spin IIC columns and centrifuged for 30s. Added 700μl from RNA washing buffer to ZYMO-spin IIC columns and centrifugation for 30s. 400μl from RNA washing buffer added to ZYMO-spin IIC columns and centrifugation for 2min. to ensure the removal of washing solution and carefully transferred ZYMO-spin IIC columns to RNAase free tubes 50μl DNAase/RNAase free water. Directly added to ZYMO-Spin IIC columns and well mixed followed by centrifugation for 30s. The ZYMO-spin IIIHRC filter was placed in collection tubes and 600μl from prep solution and centrifuged for 3min. at 8000 rpm, then RNA transferred to ZYMO-spin IIIHRC filter in RNAase free tubes and centrifuged for 3min. at 16000 rpm.

Replication of sesame plant RNA by PCR one step RT-qPCR: A replication was performed by KAPA SYBR FAST one-step qRT-PCR kit supplied from ROCHE CANDA company. A quantitative real time PCR qPCR test performed efficiently by one step (amplification and synthesis cDNA in one step) (Marisa and Juan., 2005), using RNA strand that may quickly convert. This test performed by KAPA SYBR FAST one-step qRT-PCR kit, which is a sensitive and appropriate solution in real time which includes master mix (2x) and KAPA RTmix, and using a chemical SYRBR green dye and using primers in amplification:

a. SinST1 gene: Forward:5'-GCCACAAGACACTG GACACT-3'

Reverse:5'-AGACGATCTGGCAAAGCTCC-3'

b. Sippo gene: Forward: 5'-AATCCAACCAATCGG GTCC-3'

Reverse:5'-CGACCCCGACGAGAAAGAAA-3'

c. 18sRNA (reference gene): Forward:5'-GATTCC TGCAACGAAAGCAAA-3'

Reverse:5'-CGAACACTTCACCGGACCAT-3'

Table 1: Show the required volume of each component was calculated based on the following:

Component	20 μL (Final volume)	Final concentration
Sybr green kappa Master mix	10 μL	
Forward primer	0.4 μL	0.2μM
Reverse primer	0.4 μL	0.2μM
50X KAPA RT Mix	0.4 μL	1X
Nuclease-free water	4.2 μL	
RNA Sample Volume	4.6 μL	1pg-100ng

Table 2: Show RT-PCRCycling Program.

Step	Temp. (°C)	Time	Cycle
Reverse transcription	42 °C	10 min	1
Enzyme activation	95 °C	3 min	1
Denaturation	95.0 °C	15 sec	
Annealing	53.0 °C	15 sec	40
Extension	72.0 °C	15 sec	

Table 3: Shows results of qRT-PRC technique for studied species. 1. Mahale, 2. Wadaa, 3. Rafideen and 4. Somer.

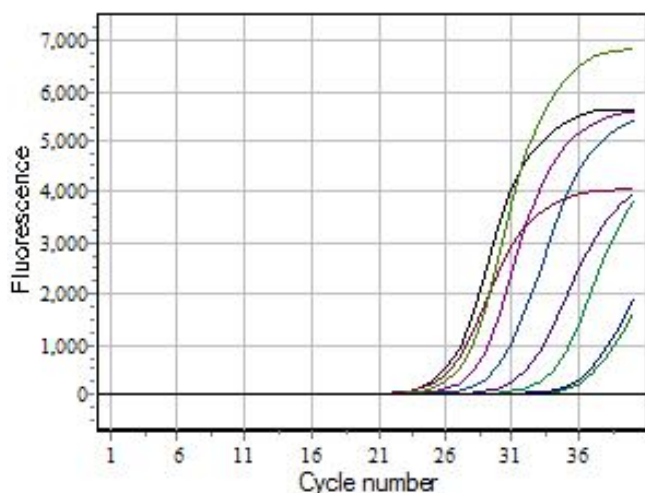| Sample Name	Ct	ΔCt	CycleNumbe- ΔCt
Sippo1	31	18	40-18=22
Sippo2	30.4	14.6	40-14.6=25.4
Sippo3	30.5	15.5	40-15.5=24.5
Sippo4	30.9	17.9	40-17.9=22.1
SiNST1-1	22	9	40-9=31
SiNST1-2	23.2	7.4	40-7.4=32.6
SiNST1-3	23.1	8.1	40-8.1=31.9
SiNST1-4	22	9	40-9=31
18sRNA1	13		
18sRNA2	15.8		
18sRNA3	15		
18sRNA4	13		

Fig. 1: Show the curves of threshold values (CT) in qRT-PRC for Sippo and SinSTi genes.

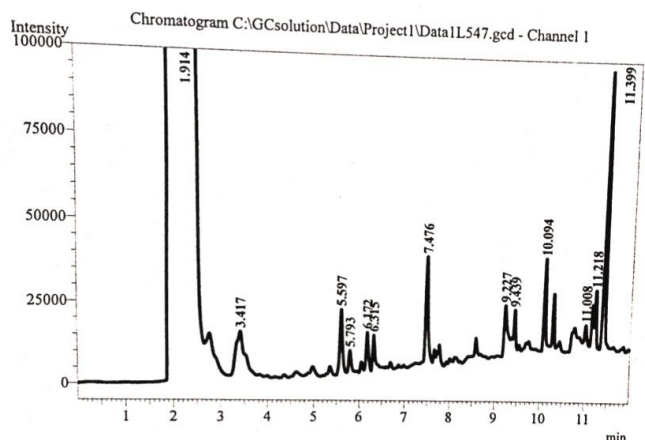**Fig. 2:** Show the analysis of fatty acids in Mahale species using GC technique.

Oil extraction: Sesame oil was extracted by soxhelt extractor, where 5 gm of dry grinded seeds samples was taken and placed in Solvent extractor. The oil extraction performed in Hexan and recorded a weight of samples after the solvent evaporated and calculated the oil percentage according to the following equation (A.O.C.S. 1976):

Oil percentage =(oil weight/dry sample weight)×100

Measurment of some saturated and unsaturated fatty acids in sesame seeds oil: Fatty acids was analyzed by gas chromatography (gas Co2010 shimadzue Japan) where used a ionized flame detection (IFD) and separated a saturated and unsaturated fatty acids from samples,

The concentrations of fatty acids in each species were as following :

Spices	Palmatic acid	Oleic acid	Lenolic acid	Lenolinic acid	Stearic acid	Fatty percentage
Mahale	0.0869%	0.443%	0.266%	36.289%	1.067%	38.1519%
Somer	0.056%	0.421%	0.221%	24.742%	0.271%	25.711%
Rafideen	0.065%	0.241%	0.242%	24.742%	0.812%	26.102%
Wadaa	0.093%	0.517%	0.129%	31.67%	1.444%	33.853%

Fig. 3: Show the analysis of fatty acids in Sumer species using GC technique.

Table 4: Show oil percentage in the studied species of sesame plant.

Oil Percentage %	Studied species
51	Mahale
47.16	Somer
48.45	Rafideen
50.8	Wadaa

their quantities and qualities were estimated and compared with separated standard samples on capillary separation columns SE30 at dimensions 30m* 0.25 mm. a saturated and unsaturated fatty acids that separated were calculated by the following equation (Zhang and Wang., 2015):

C sample = C(st) × A(sample)/A(St)

Determination a sesame lignin in sesame seeds oil: Functional compounds in sesame seeds oil were determined and estimated by HPLC. A functional compounds that extracted from sesame seeds oil are (sesamine, sesamoline, sesamol). Those functional compounds measured according to the following equation (Wang *et al*., 2005):

C sample = C(st)×A(sample)/A(St)

Results and Discussions

The results of real time PCR technique: RNA was successfully isolated from fresh leaves of sesame plant species by Quick RNA plant miniprep. The concentration of RNA ranged between (24-45 ɳg/µl) with purity ranged (1.6-1.8) to use in RT-qpcr technology to detect and estimate the level of gene expression of the two genes (SinST1 and Sippo) responsible for oil production in sesame plant. The 18S rRNA gene used as reference gene. The relative quantitative expression method is adopted where data are provided for a reference gene and expressed for
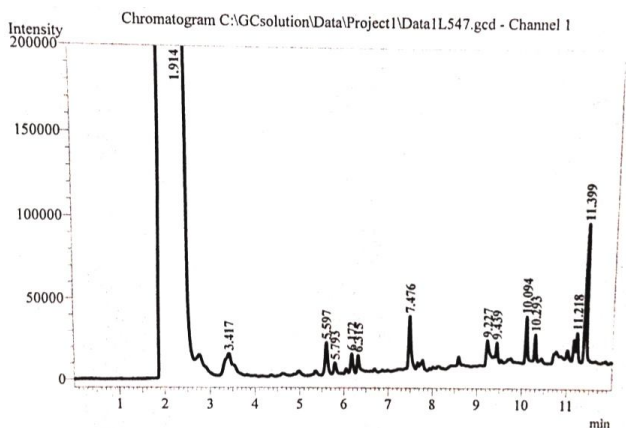
Fig. 4: Show the analysis of fatty acids in Rafideen species using GC technique.
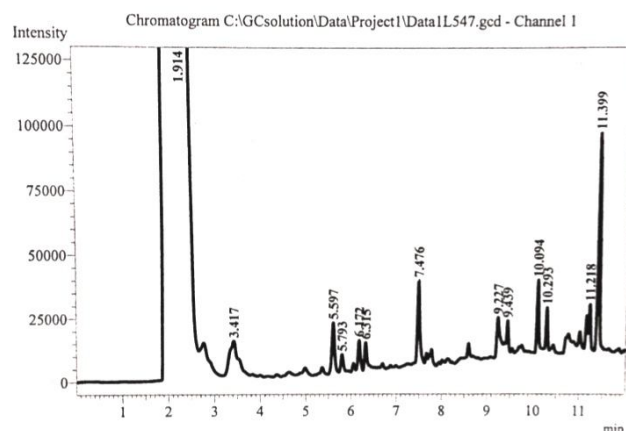

Fig. 5: Show the analysis of fatty acids in Wadda species using GC technique.

reference gene in all cells of the organism under natural and pathological conditions, Although some of reference gene is expressed in most cases at constant level, but there are genes whose expressed may change according to conditions. To compare RNA transcription levels, Ct threshold values were compared, where Ct is the number of cycles needed to fluorescence emission for the dye to reach the threshold level and detect the reaction. After determining the Ct values, a value of ΔCt is found to correct the concentration values Ct of all genes for which gene expression is determined based on the reference gene value, ΔCt is calculated from the difference between CT of the target gene and the reference gene (Xiayu *et al*., 2013) according to the following equation.

Table 5: Show the concentrations of antioxidants in species of sesame plants.

Concentrations ppm	Sesamoline ppm	Sesamine ppm	Sesamol ppm	Species
116,2	19.6	36.9	59.7	Mahale
221.2	38.8	85.5	96.9	Somer
506.6	94.6	211.8	200.2	Rafideen
269.5	80.8	88.9	99.8	Wadaa

ΔCt(patients)=Ct(patients)mean-Ct(reference)mean

The purpose of using a real time PCR is to determine a level of gene expression for target gene, the gene expression regulator level was calculated from the following equation (Libin *et al,*. 2012):

Normalized target gene expression level = cycle number - ΔCt

The amplification accuracy of gene product was noticed by the value of cycle threshold (Ct) for the triplicate reactions, the data obtained from real time experiments were detected according to the Ct values which calculated from cycles and was proportional to the starting target copy number (logarithmic scale) used for amplification (the point that the fluorescence signal increased above baseline is the threshold cycle) which are inversely related to the amount of starting template that mean the high value of Ct refers to the low levels of gene expression or amplification gene.

The highest gene expression of *SiNST1* gene in a Mahale and Wadaa varieties followed by Rafideen then somer, while a highest gene expression of Sippo gene was in Somer then Rafideen followed by Wadaa, Mahale as shown in table 1.

The results of GC technique: five fatty acids were identified, two saturated and three unsaturated fatty acid based on a standard solution.

a. Saturated fatty acids

1. Palmatic Acid, 2. Stearic Acid

b.. Unsaturated fatty acids

1. Oleic Acid, 2. lenolic Acid, 3. Lenolinic Acid

As shown in the table above, the highest percentage of fatty acids studied was in the Mahale variety and the lowest was in the Sumer variety.

As shown in the table above, the highest percentage of oil was in the Mahale variety and the lowest was in the Somer variety.

The results of HPLC technique: the results of HPLC technique showed that there are sesame lignin in oil of sesame plants: sesamol, sesamoline and sesamine according to standard solution and analysis conditions.

As shown in table 5, the highest concentration of ginans was in the Rafidain variety and the lowest concentration was in the local cultiva.

The highest level of gene expression of *SiNST1* gene was record in Mahale and Wadaa Varieties. The highest percentage of oil was found in Mahale followed by Wadaa, then the Rafideen variety where the level of gene expression and oil ratio was higher than the Somer variety,
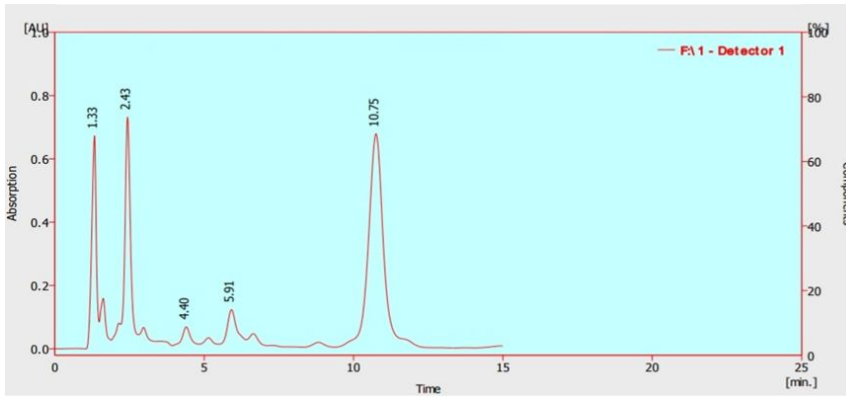
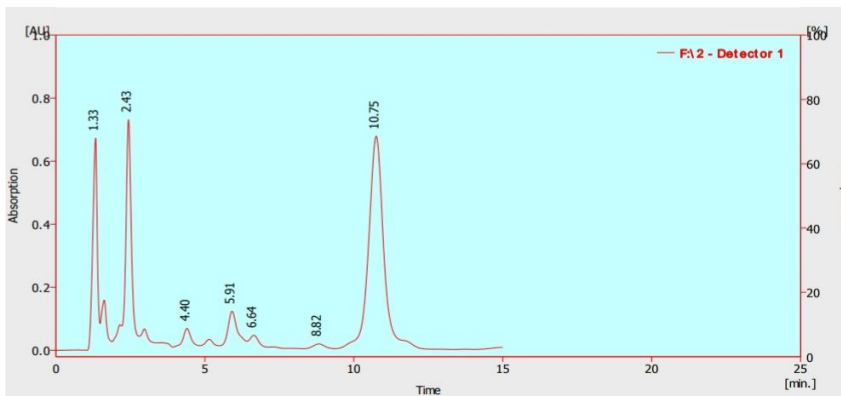**Fig. 6:** Show the analysis of sesame lignin in Mahale species.

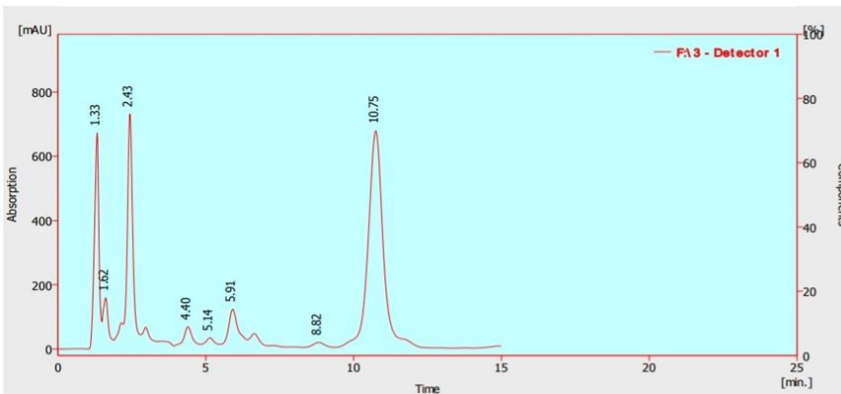**Fig. 7:** Show the analysis of sesame lignin in somer species

Fig. 8: Show the analysis of sesame lignin in Rafideen species
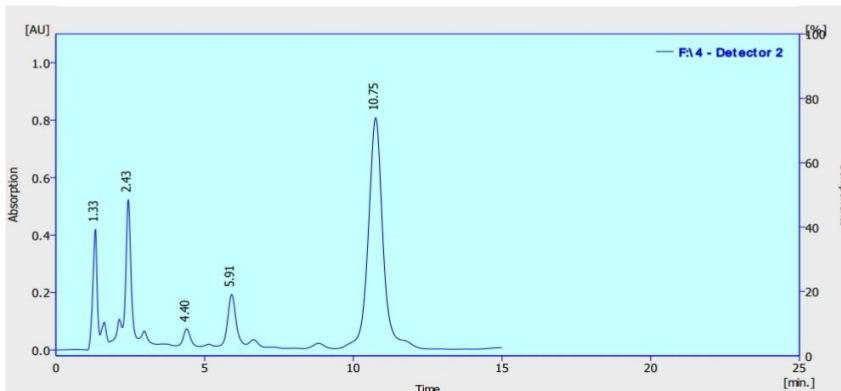

Fig. 9: Show the analysis of sesame lignin in Wadaa specie.

the level of gene expression and oil percentage in somer was lower than other varieties, this indicates to the direct correlation between expression level of *SiNST1* gene and oil percentage (where a gene expression increase, the oil percentage increase), a level of gene expression of *SiNST1* gene effects on the percentage of fatty acids whereas, a level of gene expression increase the percentage of fatty acids (lenolinic, Stearic, oleic, lenolic and palmatic acid) increase too.

The *SiPPO* gene showed that a lowest level of gene expression was found in Mahale varieties while the oil percentage was higher than in it compared to the other varieties followed by Wadaa, where the level of gene expression was Lower than somer and rafideen while the oil percentage was found higher than rafideen and somer. The highest level of gene expression in somer but the oil percentage was lower than other species. This indicate to inverse relationship between gene expression of Sippo gene with oil percentage (when expression level decrease leading to increase in oil percentage). The level of gene expression of Sippo gene effected on fatty acids percentage whereas the level gene expression increase, the percentage of fatty acids decrease. It's founded that there are a relationship between oil percentage and it percentage of fatty acids. This difference between oil and fatty acids percentage is attributed to a variation of genetic and environmental factors. The study of gene expression levels for *SiPPO* and *SiNST1* found a variation in level of gene expression for each varieties. This variation in expression may be caused by a mutations. The gene expression based on a set of molecular mechanisms that affected on different steps of mRNA synthesis which include transcription, treatment, binding, transport, translation and storage. The gene expression is an essential level at which a genotype is

expressed into phenotype that mean a genetic codon in DNA is translated to phenotype according to gene expression by proteins synthesis that control of the organism shape or acting as enzymes which stimulate the synthesis pathways of compounds within cells. The expression of genes also influenced by internal and external environmental factors. The internal factors are hormones and metabolic processes, while the external are any factors which effect on gene expression such as amount of light, the environment that plant grown in it (amount of plants in area unit) where it's affected on a light, nutrient, temperature and water amount.

The highest concentrations of Sesame lignans (antioxidants) was found in Rafideen variety followed by Wadaa, somer and Mahale varieties. The *SiNST1* gene was highest gene expression in the Mahale variety, while the concentration of Sesame lignan (antioxidant) was lower than all varieties, followed by the Wadaa variety the level of gene expression was higher while concentration of Sesame lignan (antioxidant) lower than Rafideen variety, followed by the Rafideen variety the level of gene expression was lower than Mahale and wadaa varieties while the concentration of Sesame lignan (antioxidant) higher than all varieties, Finally, somer variety the level of gene expression was lower than all varieties while the concentration of Sesame lignan (antioxidant) higher than Mahale variety. The *SiPPO* gene was the highest expression in the Somer variety while the concentration of Sesame lignan (antioxidant) was lower than Wadaa and rafideene varieties, followed by the Rafideen variety the level of gene expression was lower than the Somer variety while the concentration of Sesame lignan (antioxidant) was higher than all varieties, followed by Wadaa variety the level of gene expression was Less than Somer and Rafideen varieties while the concentration of Sesame lignan (antioxidant) is lower than Rafideen variety and higher than Somer variety and finally Mahale variety the level of gene expression was lower than all varieties as well as the concentration of Sesame lignan (antioxidant) less than all varieties, this indicates that there is no relationship between genes and the content of Sesame lignans (antioxidants) or that there is no relationship between the proportion of oil and the content of Sesame lignan (antioxidant).

The results showed that was a direct correlation between gene expression of *SiNST1* gene and oil percentage, whereas *SiPPO* gene showed an inverse relationship between a level of gene expression and oil percentage. In addition, the *SiPPO* and *SiNST1* haven't effect on Sesame lignans, also, there was not a relationship between oil percentage and Concentration of Sesame lignans.

References

A.O.C.S. (1976). Offcial and tentative method of American oil chemists crud fat Aa 6-38. Free Gossypol Ba 7-58. Moisture Ba 2-38. *The Societ Champain*, TL.

Abdalbasit, A.M., M.A. Yousif and M.O. Nuha (2014). Composition and Stability of Traditional Processed Sesame oil . *Innovative Romanian food Biotechnology*., **15:** 51-57.

Abeer, F.R., H.H. Imad and J.K. Mohanad (2017). A Review: Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Natural Compounds of Some Plants. *International Journal of Toxicological and Pharmacological*., **9(1):** 81-85.

Abuo-Gharbia, H.A., A.A.Y. Shehata and F. Shahidi (2000). Effect of processing on oxidative stability and lipid classes of sesame oil. *Food Res. Int.,* **33:** 331-340.

Ajay, P., S.B. Amarinder and K. Farhath (2010). Nutritional, Medicinal and industrial Uses of Sesame (*Sesamum indicum* L.) Seeds-AN Overview. *Agriculturae Conspectus Scientificus*., **75(4) :** 159-168.

Ashakumary, L., I. Rouyer, Y. Takahashi, T. Ide, N. Fukuda, T. Aoyama, T. Hashimoto, M. Mizugaki and M. Sugano (1999). Sesamin, a sesame Lignan, is a potent inducer of hepatic fatty acid oxidation in rat. *Metabolism*., **48:** 1303-1313.

Bustin, S.A., V. Benes, T. Nolan and M.W. Pfaffl (2005). Quantitative real-time RT-PCR- a perspective. *J. Mol. Endocrinol.,* **34:** 597-601.

Cheng, F.C., T.R. Jinn, R.C. Hou and J.T.C Tzen (2006). Neuroprotective effect of sesamin and sesamolin on gerbil brain in cerebal ischemia. *Int. J. Biomed. Sci*., **2:** 284-288.

Cheung, S.C., Y.T. Szeto and I.F. Benzie (2007). Antioxidant Protection of edible oils. *Plant Foods Hum Nutr*., **62 :** 39-42.

Dachtler, H.M., F.V. Frans and C.M. Stijin (2003). Characterization of sesame oil extracts and assessment of their antioxidant activity. *Eur. J. Lipid. Sci. Technol*., **105:** 488-496.

Libin, W., M. Hongmei, Z. Ruihong, H. Xiuhua, Z. Tide and Z. Haiyang (2012). Identification and testing of reference genes for Sesame gene Expression analysis by quantitative real-time PCR. *Plantat*., **28:** 597-604.

Marisa, L.W. and F.M. Juan (2005). Real-time PCR for mRNA quantitation. *Bio. Techniques*., **39(1):** 75-85.

Monteiro, E.M., L.A. Chibli, C.H. Yamamoto, M.C. Pereira, F.M. Vilela, M.P. Rodarte, M.A. Pinto, M.D. Amaral, M.S. Silverio, A.L. Araujo and O.V. Sousa (2014). Antinociceptive and anti-inflammatory activities of the sesamin. *Nutrients.,* **6(5) :** 1931-1944.

Nakano, D., D. Kurumazuka, Y. Nagai, A. Nishiyama, Y. Kiso and Y. Matsumura (2008). Dietary sesamin suppresses aortic NADPH oxidase in DOCA Salt hypertensive rats. *Clin. Exp. Pharmacol. Phsiol*., **35 :** 324- 326.

Nzikou, J.M., L. Matos, G. Bouanga-Kalou, C.B. Ndangui, N.P.G. Pambou-Tobi, A. Kimbonguila, T. Silou, M. Linder and S.

Desobry (2009). Chemical Composition on the seeds and oil of sesame (*Sesamum indicum* L .) grown in Congo - Brazzaville. *Advance Journal of and Food Science Technology.,* **1(1):** 6-11.

Robert, F.W. (2008). Molecular Biology. 4th edition, McGraw Hill International edition. Textbook, Univ. of Kansas. USA.

Schwertner, H.A. and D.C. Rios (2012). Analysis of sesamin, asarinin and sesamolin by HPLC with photodiode and fluorescent detection and by GC/MS: Application to sesame oil and serum samples. *J. Am. Oil Chem. Soc.*, **89:** 1943-1950.

Sirato-Yasumoto, S.M., Y. Katsuta, Y. Okuyama and T. Takahashi Ide (2001). Effect of Sesame Seeds rich in Sesamin and Sesamolin on Fatty acid oxidation in rat liver. *J. Agri. food Chem.*, **49:** 2647-2651.

Wang, L.Y., P.L.X. Zhang, W. Wang, W. Zhang and X. Wei (2005). HPLC Analysis of seed Sesamin and Sesamolin Variation in a Sesame Germplasm collection in China. *J. Am. Oil Chem. Soc.*

Xiayu, R.A., L.A. Dejian and H.U. Xuelin (2013). A New Method For Quantitative Real-Time Polymerase Chain Reaction Data Analysis. *Journal of Computational Biology.*, **20:** 703-711.

Zhang, H.Z. and O.L. Wang (2015). Development and validation of a GC- FID method for quantitative analysis of oleic acid and related fatty acids. *Journal of Pharmaceutical Analysis.*, **5(4):** 223-230.