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

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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: Palmitic 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

Sesameum 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 (Abuogharbia 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 IICG 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 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 IICG filter was placed in collection tubes and 600µl from prep solution and centrifuged for 3min. at 8000 rpm, then RNA transferred to ZYMO-spin IICG 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 SYBR green dye and using primers in amplification:

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

Reverse: 5′-AGACGATCTGCAAGCTCC-3′
b. Sippo gene: Forward: 5′-AATCCAAGCTCAAGTGCTCC-3′

Reverse: 5′-CGAAGGTGAACTCCAAAAA-3′
c. 18sRNA (reference gene): Forward: 5′-GATTCC TGCAACGAAGAAAA-3′

Reverse: 5′-CGAAGACCTTCAACGAGGACC-3′

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

<table>
<thead>
<tr>
<th>Component</th>
<th>20 µL (Final volume)</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sybr green kappa Master mix</td>
<td>10 µL</td>
<td></td>
</tr>
<tr>
<td>Forward primer</td>
<td>0.4 µL</td>
<td>0.2 µM</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>0.4 µL</td>
<td>0.2 µM</td>
</tr>
<tr>
<td>50X KAPART Mix</td>
<td>0.4 µL</td>
<td>1X</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>4.2 µL</td>
<td></td>
</tr>
<tr>
<td>RNA Sample Volume</td>
<td>4.6 µL</td>
<td>1pg-100ng</td>
</tr>
</tbody>
</table>

**Table 2:** Show RT-PCR Cycling Program.

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp. (°C)</th>
<th>Time</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse transcription</td>
<td>42 °C</td>
<td>10 min</td>
<td>1</td>
</tr>
<tr>
<td>Enzyme activation</td>
<td>95 °C</td>
<td>3 min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95.0 °C</td>
<td>15 sec</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>53.0 °C</td>
<td>15 sec</td>
<td>40</td>
</tr>
<tr>
<td>Extension</td>
<td>72.0 °C</td>
<td>15 sec</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Ct</th>
<th>ΔCt</th>
<th>CycleNumber ΔCt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sippo1</td>
<td>31</td>
<td>18</td>
<td>40-18=22</td>
</tr>
<tr>
<td>Sippo2</td>
<td>30.4</td>
<td>14.6</td>
<td>40-14.6=25.4</td>
</tr>
<tr>
<td>Sippo3</td>
<td>30.5</td>
<td>15.5</td>
<td>40-15.5=24.5</td>
</tr>
<tr>
<td>Sippo4</td>
<td>30.9</td>
<td>17.9</td>
<td>40-17.9=22.1</td>
</tr>
<tr>
<td>SiNST1-1</td>
<td>22</td>
<td>9</td>
<td>40-9=31</td>
</tr>
<tr>
<td>SiNST1-2</td>
<td>23.2</td>
<td>7.4</td>
<td>40-7.4=32.6</td>
</tr>
<tr>
<td>SiNST1-3</td>
<td>23.1</td>
<td>8.1</td>
<td>40-8.1=31.9</td>
</tr>
<tr>
<td>SiNST1-4</td>
<td>22</td>
<td>9</td>
<td>40-9=31</td>
</tr>
<tr>
<td>18sRNA1</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18sRNA2</td>
<td>15.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18sRNA3</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18sRNA4</td>
<td>13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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):

\[
\text{Oil percentage} = \left( \frac{\text{oil weight}}{\text{dry sample weight}} \right) \times 100
\]

Measurement 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 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):

\[
C_{\text{sample}} = C_{(\text{st})} \times A(\text{sample})/A(\text{St})
\]

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):

\[
C_{\text{sample}} = C_{(\text{st})} \times A(\text{sample})/A(\text{St})
\]

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 ng/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
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):

\[ \Delta Ct(\text{patients}) = Ct(\text{patients})\text{mean} - Ct(\text{reference})\text{mean} \]

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 \( \text{SiNST1} \) 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. Palmitic 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, sesameoline and sesamine according to standard solution and analysis conditions.

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

The highest level of gene expression of \( \text{SiNST1} \) 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,
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 \textit{SiNST1} gene and oil percentage (where a gene expression increase, the oil percentage increase), a level of gene expression of \textit{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 palmetic acid) increase too.

The \textit{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 \textit{Sippo} and \textit{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) were lower 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


