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Diversity Analysis of Sesame Lignans in 40 Sesame Collections in Tamil Nadu, India

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A B S T R A C T

Sesame (*Sesamum indicum* L.) is an important oilseed crop possessing medicinal values and commonly cultivated in tropical and sub-tropical areas around the world. The therapeutic value of sesamum is attributed to lignans, a prime secondary metabolite. We used High Performance Liquid Chromatography to analyse the variation of sesame lignans especially sesamin and sesamolin, the major lignans from lignan family, in 40 sesame lines maintained in Tamil Nadu Agricultural University, Tamil Nadu. HPLC results showed that sesamin, sesamolin and total lignan contents in sesame lines ranged from 0.08 to 2.58 mg/g, 0.28 to 2.52 and 0.49 to 4.55 mg/g, respectively. Among the 40 sesame lines 50% lines stayed in range 3 to 5 mg/g in total lignin content. On an average, sesame lines with brown and black seed coat colours had highest total lignan contents than yellow or white seed coat colour sesame lines. The correlation of variation between sesame lines with different seed coat colours ranked as yellow (69%) > black (35%) > brown (30%) > white (29%). This study emphasises the need to investigate variation in lignan contents in sesame seed lines to develop superior sesame genotypes.

Introduction

Sesame belongs to the genus *Sesamum*, one of 16 genera in the family Pedaliaceae. The genus *Sesamum* comprises of about 35 wild species besides the only cultivated species, *Sesamum indicum*. *Sesamum indicum*is the major commercial source of sesame seeds and is primarily grown in Burma, India, China, Ethiopia and Sudan with 9,398,770 ha under sesame cultivation worldwide, producing 4.76 million tons (FAO, 2013).

Sesame seeds contain high oil and protein accounting to about 50% and 25% respectively. Sesame protein is rich in arginine, leucine and the sulphur containing amino acids cysteine and methionine but slightly low in the essential amino acid lysine. The fatty acid profile of sesame oil reflects linoleic acid (37-47%), oleic acid (35-43%), palmitic (9-11%) and stearic acid (5-10%) with trace amount of linolenic acid (Latif and Anwar, 2011). Sesame oil isa valuable edible oil with a composition that provides good health benefits including high level of unsaturated fatty acids and antioxidants (Bisht *et al.,* 1998). Sesame seed oil has significant antimicrobial activity against many bacteria and fungi (Hussein *et al.,* 2016). Sesame seed is highly valued for its oil which is exceptionally resistant to oxidative rancidity as compared to other vegetable oils (Hemalatha, 2004). Sesame seed contains a group of compounds collectively known as lignans, to which many health promoting effects are attributed.

Lignans, a group of secondary metabolites are responsible for the therapeutic value of sesame oil. Sesame lignans, especially sesamin and sesamolin possesses various biological activities attributing to its health promoting effects. These two compounds have been reported to have multiple pharmacological properties, such as anti-oxidant activity (Shahidi *et al.,* 2006), anti-proliferative activity (Yokota *et al.,* 2007) and anti-inflammatory function (Hsu *et al.,* 2005). In addition, anti-cholesterolemic and antihypertensive properties of sesamin have beenreported (Penalvo *et al.,* 2006). Lignans, the natural anti-oxidants in sesame oil prevent oxidative rancidity and provide high stability and improved the shelf life of the oil (Suja *et al.,* 2005). The benefits of lignans on human health being well established, it is important to select a high lignan especially sesamin and sesamolin germplasm that are ideal for edible, therapeutic and industrial purposes. The objective of present study is to investigate the content of lignans especially sesamin and sesamolin, in sesame lines maintained at Tamil Nadu Agricultural University, Tamil Nadu.

Materials and Methods

Materials and Chemicals

40 sesame lines maintained in the Department of Oilseeds, Tamil Nadu Agricultural University, Tamil Nadu were used in present study [coat colour: brown (n=23), black (n=6), yellow (n= 8), white (n=3)]. Lignan stand ards and methanol (HPLC grade) were purchased from Sigma-Aldrich. Calibration curves were prepared by injecting varying concentration of working stand ard in HPLC system.

Sample Preparation

Samples were prepared according to the method of Shi *et al.,* (2017) with some modifications. Briefly, 0.2 mg of seed sample was crushed in mortar and pestle with 5 ml of methanol. The extract was shaken vigorously for 2 minutes using a vortex mixer and sonicated for 2 minutes. Samples were then centrifuged at 10,000 rpm for 5 min. and the supernatant was collected. The residue was re-extracted with another 2 mL of methanol. The supernatants were pooled and the final volume of each extract was made up to 5mL. Prior to HPLC analysis it was filtered through a 0.45-μm nylon membrane.

HPLC Analysis

The quantitation of sesamin and sesamolin in the sesame seed was achieved by HPLC-DAD system (Agilent 1220 Infinity II LC of Agilent Technologies Co., USA) including a binary pump, an auto-sampler, temperature-controlled column oven and a diode array detector (DAD). The separation was carried out on a reversed-phase C18 column (150 mm × 4.6 mm i.d., 5 μm) under the following chromatographic conditions: the injection volume was 10 μL, column temperature was maintained at 30°C, wavelength of UV detection was set at 290 nm and mobile phase consisted of methanol and Milli-Q water (70/30, v/v) with a flow rate of 1.0 mL/min. Data analysis was performed in Excel and Statistical Package for the Social Sciences (SPSS) 16.

Results and Discussion

Forty sesame lines collections maintained in Tamil Nadu Agricultural University, Tamil Nadu were analysed by HPLC for separation of isolated lignans especially sesamin and sesamolin, the major lignans from lignan family. The HPLC system yielded two fractions at 9.5 min. and 12.5 min. corresponding to sesamin and sesamolin respectively (Fig. 1). The sesamin and sesamolin content in 40 sesame lines are listed in Table 1. The present study reveals that the total lignan (sesamin and sesamolin) contents of these lines ranged from 0.49 mg/g to 4.55 mg/g with an average of 2.69 mg/g. The sesamin content of these sesame lines ranged from 0.08 mg/g to 2.58 mg/g with an average of 1.28 mg/g. The sesamolin content of these lines ranged from 0.28 mg/g to 2.52 mg/g with an average 1.41 mg/g. The total ligancontent reported in the present study is higher than the values reported earlier in sesame lines of Tamil Nadu (Pathak *et al.*, 2015). The highest total lignan content was recorded in sesame line KMR-108 (4.55 mg/g), a brown seed coat line followed by SI-3106 (4.48 mg/g), SI-1236 (4.17 mg/g). Distribution of 40 sesame lines according to their lignin contents are shown in Figure 2. The distribution result showed about 50% of sesame lines stayed in the range of 3 to 5 mg/g. Among these only four sesame seed lines stayed in the range of 4.0-5.0 mg/g (KMR-108, SI-3106, SI-1236 and SI-861/2).

According to the seed coat colours, we divided the 40 sesame seed lines in to four groups as brown, black, yellow and white. A one way ANOVA (between groups) showed the sesame seeds with brown seed coat had significantly high sesamin and sesamolin content than those with black, yellow or white seed coat (P= <0.05). Coefficient of Variation (CV) was used to study the variation in sesamin and sesamolin among the different seed coat colours. The results indicate that yellow (71%) > white (52%) > brown (36%) > black (32%). The yellow seed coat sesame lines had more variation as compared to the other seed coat colours and yellow (73%)>black (41%) > brown (29%) > white (2%). With respect to sesamolin also yellow seed coat line exhibited more variation than sesame lines of other colours. The results of CV indicate that the sesame lines with yellow seed coat colour had more variation than those with brown, black or white seed coat. Coefficient of Correlation (CC) between sesamin and sesamolin indicated positive correlation by Pearson's correlation coefficient. Wang *et al.*, (2013) showed darker seed coat colour sesame had highest positive correlation, as black (R= 0.96) showed highest CC for sesamin and sesamolin than those with yellow and white. Interestingly in the present study we observed highest CC in yellow seeded line for sesamin and sesamolin (R= 0.80) than brown, black or white.

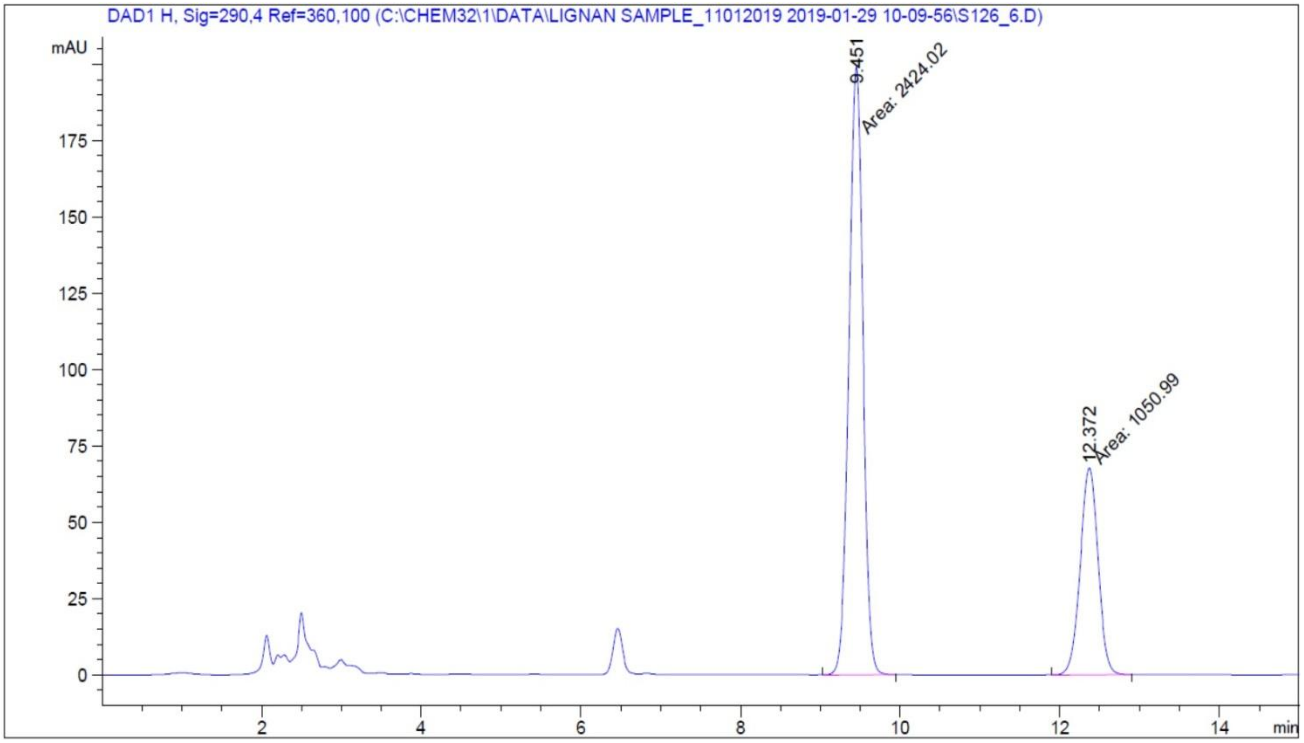
The furofuran type of sesame lignan has innumerable beneficial effects on human health. The health promoting effects of sesame seeds in human body may be attributed to sesamin contents. Sesame lignan isolation attempts, especially sesamin and sesamolin have been made ever since 1940 when the synergistic effect of sesame oil for the insecticidal pyrethrins was reported (Tracy, 1958).

After that, the main focus has been made to isolate two of the major aglyconlignans especially, sesamin and sesamolin from varied sources (Hemalatha *et al.,* 2004; Wang *et al.*, 2012; Shi *et al.*, 2017). Sesame lignan stimulate hepatic fatty acid oxidation by affecting the gene expression of various proteins regulating hepatic fatty acid oxidation (Ide *et al.*, 2009).

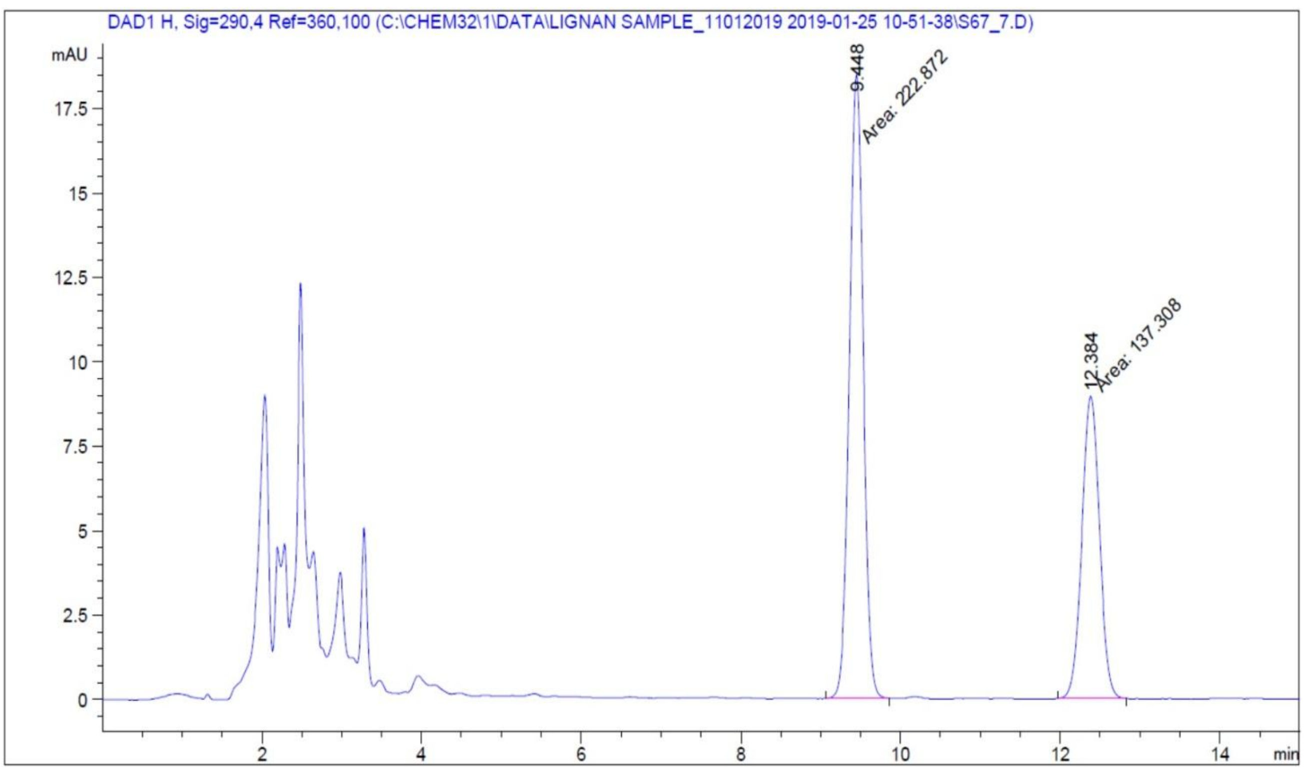
Table.1 The lignan contents in 40 sesame lines

Name	Colour	Sesamin (mg/g)	Sesamolin (mg/g)	Total Lignan (mg/g)
IS-621	Br	1.24	1.26	**2.50**
KMR-95	Br	1.08	1.34	**2.42**
SI-1885	Br	1.59	2.06	**3.65**
SI-608	Br	0.71	0.79	**1.51**
SI-3106	Br	1.96	2.52	**4.48**
SI-4721	Br	1.81	1.66	**3.46**
IC-1994-38	Br	1.82	1.71	**3.53**
AST-5	Br	1.45	2.12	**3.58**
KMR-80	Br	1.78	2.15	**3.93**
SI-1769	Br	0.46	1.17	**1.63**
KMR-108	Br	2.58	1.98	**4.55**
DS-1	Br	1.82	1.90	**3.71**
SI-861/2	Br	1.87	2.18	**4.05**
BS-27	Br	1.48	1.20	**2.68**
SI-1657	Br	0.17	0.64	**0.81**
ES-13	Br	1.58	1.56	**3.14**
SI-3278/2	Br	1.95	1.51	**3.46**
SI-30-1	Br	1.77	1.58	**3.35**
SI-1861/2	Br	1.37	1.79	**3.17**
SI-9185	Br	0.94	1.03	**1.97**
SI-1818	Br	1.79	1.37	**3.16**
KMR-69	Br	1.82	1.32	**3.15**
SI-771	Br	1.45	1.75	**3.20**
SI-1236	B	1.82	2.35	**4.17**
SI-70	B	0.76	0.84	**1.59**
SP-7613	B	1.74	2.25	**3.99**
SI-837	B	0.98	1.06	**2.04**
PAIYUR-1	B	1.81	1.17	**2.99**
TKG-84	B	1.67	2.06	**3.73**
JLS-57	Y	0.24	0.26	**0.49**
SI-1804	Y	1.15	1.46	**2.61**
NIC-8261	Y	0.61	1.64	**2.24**
SO-233	Y	0.34	0.38	**0.72**
SI-7212	Y	0.78	0.82	**1.60**
NIC-1610	Y	1.58	2.37	**3.95**
DCP-1810	Y	0.82	0.51	**1.32**
SI-1712	Y	0.08	0.64	**0.72**
KMR-7	W	0.55	0.65	**1.19**
ESN-32	W	1.35	0.63	**1.99**
TC-25	W	0.65	0.66	**1.30**

Fig.1 HPLC separation of lignans in sesame lines a) KMR-108 and b) JLS-57

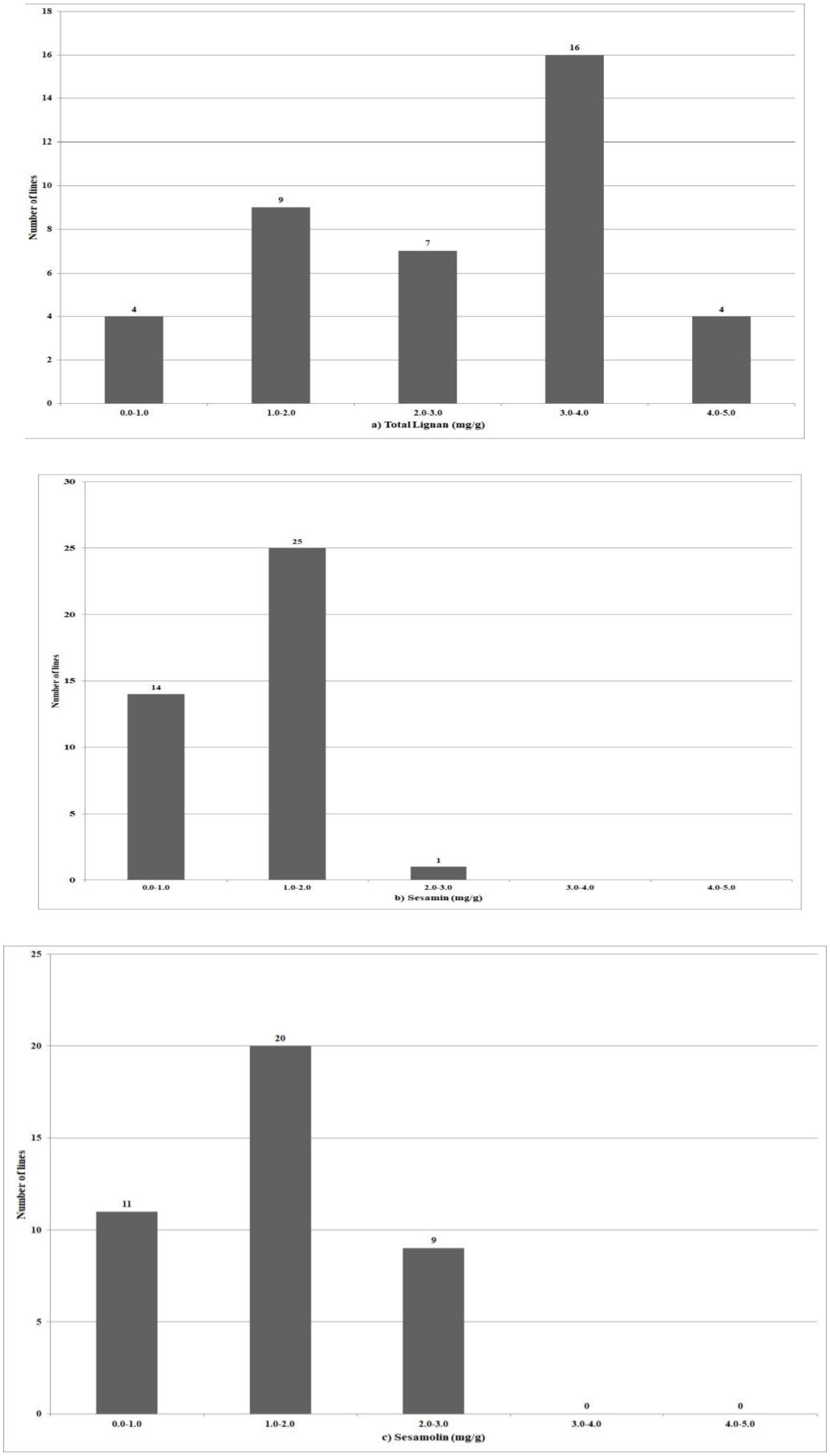


a) KMR-108



b) JLS-5

Fig.2 Distribution plot for content of a) total lignan, b) sesamin and c) sesamolin among in 40 sesame lines



Sesame lignan contributes to the lowering of triglycerides in blood by promoting fatty acid oxidation. Anti-aging property of the isolated lignans was studied using *Caenorhabditis elegans* as a relevant animal model to develop anti-aging agents (Su *et al.*, 2015).

Phitak *et al.*, (2012) opined that "sesamin may be a natural drug of choice for treatment of arthritic diseases". Intervention and epidemiological studies on dietary intake of lignans in relation to cardiovascular disease risk indicated high lignan doses decreased the risk of cardiovascular disease (Peterson *et al.*, 2010). This study established the positive effect of lignans in preventing cardiovascular diseases. The associations between lignans and decreased risk of cardiovascular disease are promising, but are yet not well established, perhaps due to low lignan intakes in human diet. Our study provides valuable information on lignans especially sesamin and sesamolin contents in sesame seeds and can identify the potential germplasmlines for breeding sesame lines with high lignan contents.

In the present study we observed greater variation in sesamin and sesamolin contents in 40 sesame lines maintained in Tamil Nadu Agricultural University, Tamil Nadu. Among these KMR-108, SI-3106, SI-1236 and SI-861/2 had highest content of sesamin and sesamolin, indicating that these lines can be use as functional food. It has a high lignan value we can use it in cosmetic industries or in pharmaceutics industries etc. The lines with high lignan content may be ideal for breeding programme to evolve high oil yielding lines with high levels of sesamin and seamolin.

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