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VARIATION OF SESAMIN AND SESAMOLIN CONTENTS IN SESAME CULTIVARS FROM CHINA

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Abstract

A collection of 62 sesame cultivars from China were analyzed for sesamin and sesamolin contents in seeds using HPLC. Results showed the sesamin and sesamolin contents of these cultivars ranged from 0.82 to11.05 mg/g and 1.35 to 6.96 mg/g, respectively. About 60.0% of the cultivars stayed in the range from 6.0 to 9.0 mg/g in the total content of sesamin and sesamolin. On average, cultivars with white seed coat color had higher sesamin content than those samples with black color. The correlation coefficient between sesamin and sesamolin of the cultivars with different seed colors ranked as white (R = 0.44) < medium (R = 0.75) < black (R = 0.96). The landraces had higher sesamin and sesamolin contents than other cultivars significantly. The results of this study exhibited useful lignan information of the cultivars from China and identified potential cultivars having high sesamin or sesamolin for functional food, pharmaceutics and cosmetic industries.

Introduction

Sesame (*Sesamum indicum* L.) (2n = 26), is an important oil crop in tropical and subtropical areas and belongs to the family *Pedaliaceae* (Ashri, 2010; Akbar *et al*., 2011). Myanmar, India, China, Sudan and Ethiopia are the most important sesame producers with about 70% of world planting areas and 60% of world production (Anon., 2009). Sesame seed oil is highly appreciated for its nutritional value linked to providing health benefits. Sesame seeds are used in candies, as well as ingredients in bread, chips and health foods (Namiki, 1995). Currently, the consumption of sesame seed products and oils is steadily increasing in Europe and USA (Moazzami *et al*., 2007; Morris, 2009).

Sesame seeds contains 50-60% oil, and nearly 85% unsaturated fatty acids (Latif & Anwar, 2011). It is known to be significantly resistant to oxidative rancidity and could be kept for a long time (Abou-Gharbia *et al*., 2000; Yen & Shyu, 1989). The reasons for this superior oxidative stability remain unclear. Research has mainly been focused on its high contents of physiologically active furofuran lignans, especially the two major oil-soluble lignans, sesamin and sesamolin (Budowski & Markley, 1951; Fukuda & Nakata, 1999). The two constituents have been reported to have multiple pharmacological properties, such as decreasing blood lipids (Hirata *et al*., 1996) and arachidonic acid levels (Shimizu *et al.*, 1991), lowering cholesterol levels (Chen *et al*., 2005; Hirata *et al*., 1996; Visavadiya & Narasimhacharya, 2008), providing anti-proliferative activity (Yokota *et al*., 2007) and anti-inflammatory function (Hsu *et al*., 2005; Utsunomiya *et al*., 2000), increasing hepatic fatty acid oxidation enzymes (Ashakumary *et al*., 1999), and showing antihypertensive (Nakano *et al*., 2008) and neuroprotective effects against hypoxia or brain damage (Cheng *et al*., 2006). Sesamin is so far the most potent food known to effectively improve γ-tocopherol bioavailability (Cooney *et al*., 2001; Jiang *et al*., 2001; Lemcke-Norojarvi *et al*., 2001). Such characteristics have extended sesame utility in antiseptics, bactericides (Bedigian *et al*., 1985), functional foods, pharmaceutics and cosmetic preparations (Namiki, 2007).

Considering the advantages of sesamin and sesamolin on human health, it is desirable to select the cultivars with high contents of sesamin and sesamolin for functional foods, new cultivar breeding and other uses. Among countries planting sesame, China plays a key role in producing sesame seeds. For example, India and China grew 1,940,000 and 751,000 ha of sesame in 2003, respectively, but sesame seed yields in India were 620,000 metric tons while sesame in China yielded 825,000 metric tons (Ashri, 2007). That mainly attributes to elite cultivars planted in China. To date, more than 100 cultivars had been released in China. Before 1970s, landraces were predominant cultivars planted in China, and then pure-line cultivars (PC), radiation-induced and space-induced cultivars (IC), and pedigree selection cultivars (PSC) became prevalent for their high yield ability. Though sesame seeds and its products are used widely in China, the sesamin and sesamolin profiles of these cultivars still not clear. The objective of the present study was performed to elucidate the sesamin and sesamolin contents of China sesame cultivars.

Materials and Methods

Sesame cultivars used: The 62 sesame cultivars used in the study included 24 landraces, 6 pure-line cultivars, 5 induced cultivars, 26 pedigree selection cultivars and one hybrid cultivar (Table 1). The origins of these cultivars cover most of the sesame planting area of China, and they can be accessed from the National Medium-term Sesame Genebank of China (Wuhan, China) in a standard way. These cultivars were grown in randomized complete blocks design with three replications during 2009 growing seasons in Wuhan, Hubei province, China. Each of the trail plots comprised three rows of 2 m length spacing 40 cm with plant spacing of 10 cm. After harvest, each line was mixed together with its three replications equally and air-dried to moisture about 5.5-6.0%.

Table 1. List of sesamin and sesamolin contents in 62 cultivars.

No.	Cultivar	Category*	Release time	Release site	Coat color	Sesamin (mg/g)	Sesamolin (mg/g)	Total (mg/g)
1.	Bawangbian	Landrace	Before 1970s	Anhui	White	2.38	2.49	4.87
2.	Baitazhima	Landrace	Before 1970s	Anhui	White	8.24	5.27	13.51
3.	Muzhenbai	Landrace	Before 1970s	Anhui	Brown	11.05	6.96	18.01
4.	Miaoqianzhima	Landrace	Before 1970s	Anhui	White	5.64	3.79	9.43
5.	Dayanhei	Landrace	Before 1970s	Anhui	Black	5.38	3.96	9.35
6.	Yekezhima	Landrace	Before 1970s	Anhui	White	6.21	3.77	9.98
7.	Badacha	Landrace	Before 1970s	Henan	Brown	4.66	3.24	7.9
8.	Sitongzhima	Landrace	Before 1970s	Henan	Brown	3.58	2.64	6.22
9.	Zihuayeersan	Landrace	Before 1970s	Henan	Yellow	4.9	3.1	7.99
10.	Xiaozihuang	Landrace	Before 1970s	Henan	Yellow	4.67	4.15	8.82
11.	Liutiaoqing	Landrace	Before 1970s	Henan	Brown	4.21	3.73	7.94
12.	Heshangtou	Landrace	Before 1970s	Hubei	Brown	6.31	3.26	9.57
13.	Xiniujiao	Landrace	Before 1970s	Hubei	White	7.63	3.64	11.26
14.	Zhuganqing	Landrace	Before 1970s	Hubei	White	4.85	3.48	8.33
15.	Bagucha	Landrace	Before 1970s	Hubei	White	7.9	3.98	11.88
16.	Wujiaozhan	Landrace	Before 1970s	Hubei	White	6.67	3.58	10.25
17.	Zhuanzhulian	Landrace	Before 1970s	Hubei	White	8.71	2.74	11.44
18.	Yibabai	Landrace	Before 1970s	Hubei	White	5.98	3.66	9.64
19.	Qiancengta	Landrace	Before 1970s	Jiangxi	Black	4.78	3.4	8.18
20.	Zichangma	Landrace	Before 1970s	Jiangxi	Brown	6.95	2.15	9.1
21.	Wuninghei	Landrace	Before 1970s	Jiangxi	Black	5.46	3.55	9
22.	Loushanglou	Landrace	Before 1970s	Shanxi	White	7.81	3.44	11.26
23.	Wucuolian	Landrace	Before 1970s	Shanxi	White	8.54	3.94	12.48
24.	Zhuanjiaolou	Landrace	Before 1970s	Sichuan	Brown	2.84	2.56	5.39
25.	Jizhi No.1	PSC	1974	Hebei	White	3.24	3.08	6.32
26.	Zhongzhi No.7	PSC	1982	Henan	White	6.01	2.7	8.71
27.	Liaozhi No.1	PC	1983	Liaoning	White	4.71	3.92	8.63
28.	Jizhi No.3	PSC	1987	Hebei	White	3.69	3.07	6.77
29.	Yuzhi No.4	PSC	1992	Nationwide	White	4.48	2.98	7.46
30.	Jinzhi No.2	PC	1995	Shanxi	White	4.77	3.68	8.45
31.	Ji 9014	PSC	1998	Hebei	Black	0.82	1.87	2.69
32.	Ezhi No.1	PSC	1998	Hubei	White	6.12	2.59	8.71
33.	Yuzhi No.11	IC	1999	Henan	White	4.1	2.93	7.03
34.	Ezhi No.2	PSC	2000	Hubei	White	4.94	2.26	7.2
35.	Zhongzhi No.12	PSC	2003	Hubei	White	5.89	3	8.89
36.	Zhuzhi No.11	PSC	2003	Nationwide	White	5.08	3.42	8.5
37.	Zhongzhi No.11	IC	2003	Nationwide	White	5.42	3.3	8.71
38.	Zhengzhi 98N09	PSC	2004	Nationwide	White	4.31	3.24	7.55
39.	Liaopinzhi No.2	PC	2004	Liaoning	Black	5.33	3.28	8.61
40.	Jinzhi No.3	IC	2004	Shanxi	Black	0.94	1.35	2.29
41.	Ezhi No.3	PSC	2005	Hubei	White	6.7	3.03	9.74
42.	Luozhi No.18	PSC	2005	Nationwide	White	5.18	3.24	8.41
43.	Zhuzhi No.14	PSC	2005	Nationwide	White	4.67	2.7	7.38
44.	Zhongzhi No.13	IC	2005	Nationwide	White	4.79	2.84	7.63
45.	Wanzhi No.1	PSC	2006	Anhui	White	4.12	2.62	6.74
46.	Jiheizhi No.1	PSC	2006	Hebei	Black	3.22	2.88	6.1
47.	Luozhi No.16	PC	2006	Nationwide	White	5.17	3.12	8.29
48.	Hezazhi No.1	HC	2006	Anhui	White	4.46	2.93	7.38
49.	Zhengzhi No.12	PSC	2007	Henan	White	4.91	3.63	8.53
50.	Ezhi No.4	PSC	2007	Hubei	White	6.13	3.92	10.06
51.	Ezhi No.5	PSC	2007	Nationwide	White	7.69	2.39	10.08
52.	Zhongzhi No.14	PSC	2007	Nationwide	White	5.27	3.18	8.45
53.	Zhuzhi No.15	PSC	2007	Nationwide	White	6.81	3.6	10.41
54.	Luozhi No.15	PC	2007	Henan	White	4.46	3.03	7.5
55.	Wanzhi No.2	PSC	2008	Anhui	White	3.74	2.58	6.32
56.	Ganzhi No.6	PSC	2008	Jiangxi	Black	3.19	2.48	5.68
57.	Ganzhi No.7	PC	2008	Jiangxi	Black	3.68	2.6	6.28
58.	Lezhi 08	PSC	2009	Anhui	Black	1.4	1.84	3.24
59.	Zhongfengzhi No.1	PSC	2009	Anhui	White	7.14	3.35	10.49
60.	Zhengzhi No.13	PSC	2009	Henan	White	4.33	3.25	7.58
61.	Zhuzhi No.16	PSC	2009	Henan	White	3.74	2.92	6.66
62.	Hangzhi No.2	IC	2009	Anhui	White	5.65	3.04	8.69

*PC: Pure-line cultivar; IC: Radiation-induced or space-induced cultivar; PSC: Pedigree selection cultivar; HC: Hybrid cultivar

Samples preparation: Samples were extracted according to Rangkadilok *et al*., (2010) and Amber *et al.*, (2012) with modification. Briefly, 2 g of each sample were ground to a fine powder with an electric mill and passed a 0.25 mm screen. The flour was accurately weighed (200 mg) into a 10 ml cuvette and dissolved in 5.0 ml of 80% ethanol. Vortex-mixed samples were centrifuged 5 min at 17,500 rpm/min. The supernatant was transferred into a 10 ml volumetric flask and the residue was re-extracted with 5.0 ml of 80% ethanol. All extracted solutions were combined and filtered with a 0.45 µm Nylon membrane (Chrom Tech, Apple Valley, MN) prior to HPLC analysis.

HPLC analysis: The samples were analyzed according to methods described by Shahidi *et al*., (2006) and Kaya *et al*., (2012) for sesamin and sesamolin contents, using external standard method by Agilent 1100 high-performance liquid chromatography (HPLC, Agilent Technologies, Waldbronn, Germany) with a thermostatically controlled column oven, a binary pump, and a diode-array detector. A reversed-phase column, Hypersil BDS C18 5 µm, 150 × 4 mm i.d. (Thermo Electron Co., Southend-on-Sea, UK), was used in this study. The mobile phase was a mixture of methanol-deionized water (80/20, v/v) at a flow rate of 0.8 ml/min (injection volume 10µl). Absorption at 290 nm was monitored. Each sample was repeated twice, and average of two values was counted as final sesamin or sesamolin contents. If the discrepancy of the two values was 10% higher of their average, the analysis was repeated.

Results and Discussion

The 62 cultivars from China were analyzed with HPLC for sesamin and sesamolin contents. The retention time of two lignan constituents were about 4.6 and 5.6 minutes, respectively (Fig. 1). The values of two constituents were listed in Table 1. It showed the total of sesamin and sesamolin of these cultivars ranged from 2.29 to 18.01 mg/g with average 8.39 mg/g in seeds, and about 60.0% of cultivars stayed in the range from 6.0 to 9.0 mg/g. The sesamin content ranged from 0.82 to 11.05 mg/g with average 5.19 mg/g (Fig. 2), and its coefficient of variation (*CV*) was 35.6%. These values were higher than sesames from multi-countries (Tashiro *et al.*, 1990) and France (Moazzami *et al*., 2006b). Rangkadilok *et al*., (2010) also found a sesame accession from China had sesamin content greater than those from Thailand. The highest sesamin content was found in cultivar Muzhenbai, a landrace from Anhui province, followed by Zhuanzhulian (8.71 mg/g), Wucuolian (8.54 mg/g) and so on.

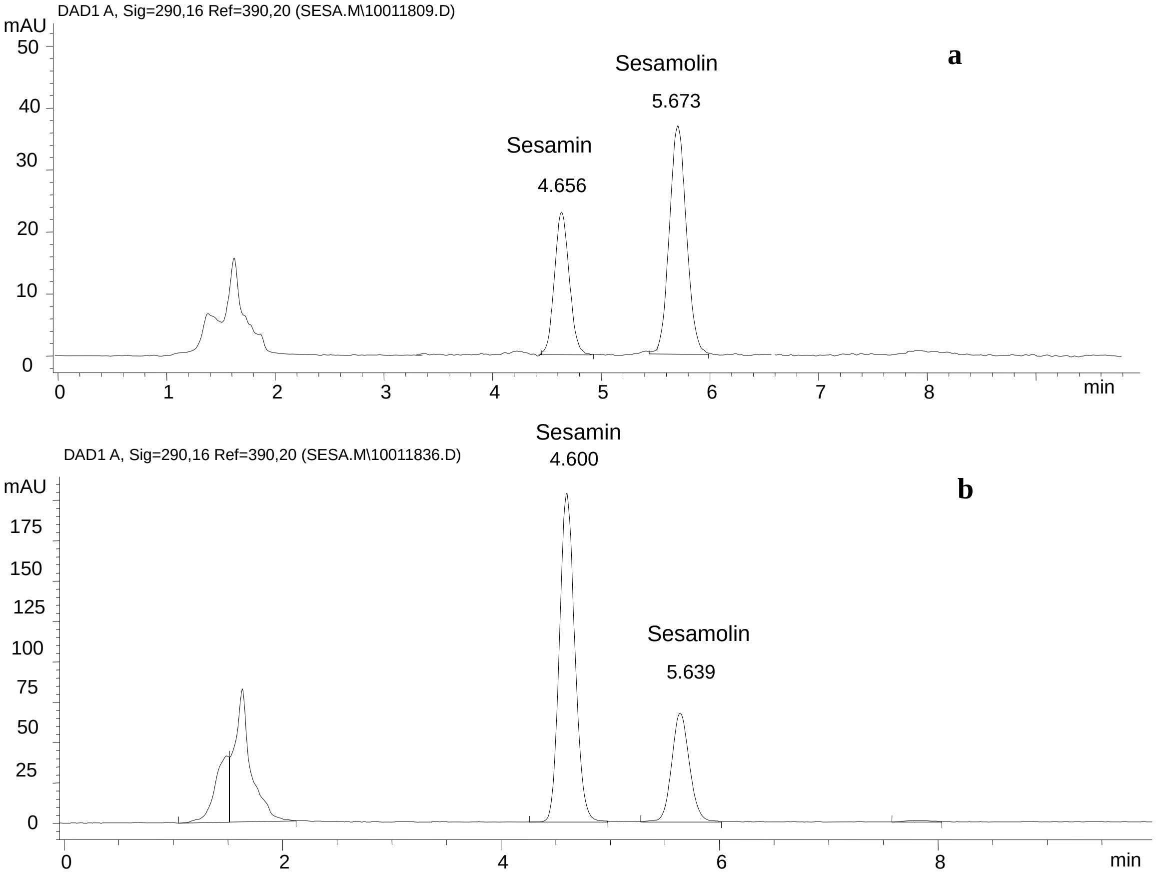

Fig. 1. The HPLC fingerprints of sesame cultivars Ji 9014 (a) and Zhongfengzhi No.1 (b)

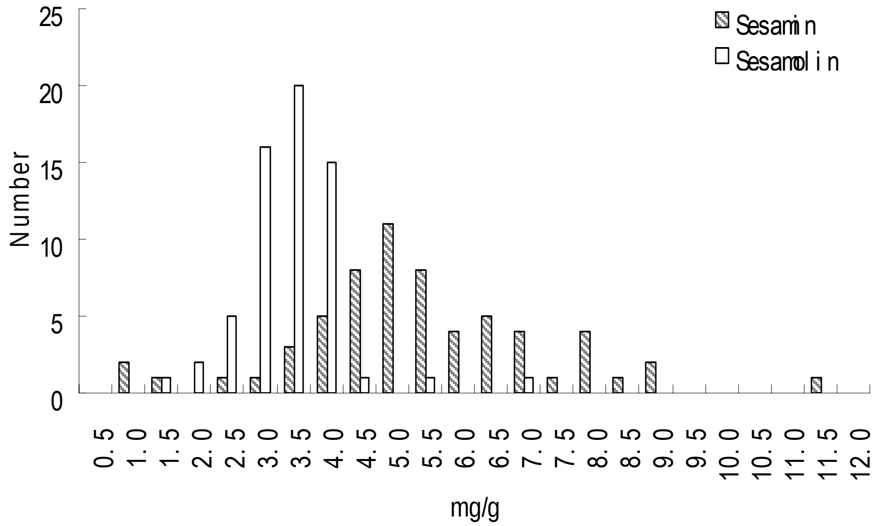

Fig. 2. Distributions of the sesamin and sesamolin contents in the 62 cultivars.

Unlike the results of Hemalatha *et al*., (2004) that sesamin and sesamolin contents were similar in sesame seeds, the sesamolin content of these cultivars from China was a little lower than sesamin on average (3.60mg/g) with *CV* 24.9%, but it was still higher than those reported previously (Moazzami *et al*., 2006b; Tashiro *et al*., 1990). The cultivar with the least sesamolin was Jinzhi No. 3 (1.35 mg/g) from Shanxi province, and highest one was also the cultivar Muzhenbai with 6.96 mg/g. Though sesame lignan mainly consists of sesamin and sesamolin (Rangkadilok *et al*., 2010), and clear peaks of the two constituents were detected here, it was believed other minor constituents, sesaminol di- and triglucosides, sesamolinol diglucoside, and pinoresinol mono-, di-, and triglucosides etc., were parts of these samples (Katsuzaki *et al*., 1994; Moazzami *et al.*, 2006a,b; Rangkadilok *et al*., 2010), as some minor peaks were observed (Fig. 1). However, their amounts were not measured for lack reference standard.

According to the seed coat color, these cultivars could be divided in to three classes as black, medium (yellow plus brown) and white. A one way ANOVA showed the cultivars with white seed coat color were higher than those with black color in sesamin and total (sesamin plus sesamolin) contents significantly ($p≤0.05$), but nonsignificant differences were detected between medium color and others. Similar results were also reported by Tashiro *et al*., (1990). However, it was not compatible with results of Moazzami *et al.*, (2006a). Though the role of the seed coat color is not clear in sesame lignan biosynthesis pathway, the present study found the sesame seeds with darker coat colors truly had a higher correlation coefficient of sesamin and sesamolin as white (R = 0.44) < medium (R = 0.75) < black (R = 0.96). Moazzami *et al.*, (2006a) found the correlation between sesamin and sesamolin was stronger for black (R = 0.88) than white (R = 0.81). Tashiro *et al*., (1990) found the white and black seed strains differed significantly in sesamin content.

The landrace Muzhenbai with brown seed coat color showed both highest sesamin and sesamolin contents, which provided evidence that sesamin synthesizes does not compete with accumulation of sesamolin. This finding partially supported the hypothesis that sesamin and sesamolin share similar biosynthesis pathway (Kato *et al*., 1998; Marchand *et al*., 1997), but it not mean that sesame with high sesamin always has high sesamolin contents, as the ratio of sesamin vs. sesamolin ranged from 0.44 to 3.23 with average 1.62. Overall, there were five cultivars, about 7.5% of total cultivars, had less sesamin than sesamolin, and 17.7% of cultivars had sesamin content twice more than sesamolin, which suggested sesamin was a predominant constituent rather than sesamolin in sesame ordinarily (Fig. 3).

Among 62 cultivars, sesamin contents of the 24 landraces ranged from 2.38 to 11.05 mg/g with average 6.05 mg/g, and the sesamolin contents varied from 1.35 to 6.95 mg/g with average 3.60 mg/g. The other cultivars varied from 0.82 to 7.69 mg/g with average 4.64 mg/g in sesamin and 1.35 to 3.92 mg/g averaged 2.94 mg/g in sesamolin. Multiple comparisons (*LSD*) indicated both sesamin and sesamolin contents of landraces were higher than that of others significantly ($p≤0.01$). Regarding released time, the 62 cultivars could be classified into three groups as Before 1970s (landraces), 1970-1999 and 2000-2009. It showed the group Before 1970s was higher than those of 1970-1999 and 2000-2009 in sesamin and sesamolin contents, but nonsignificant difference was detected between groups of 1970-1999 and 2000-2009. The reasons may be under the narrow genetic foundation of these recent cultivars, as most pure-line cultivars and pedigree selection cultivars originated one or two released cultivars formerly, which usually characterized typical high yield traits.

Identification of genetic variation is indispensable for effective management and use of genetic resources and critical to success of breeding programs (Hoisington *et al*., 1999; Rao, 2004). Here, the landraces Muzhenbai, Baitazhima, Wucuolian, Bagucha and Zhuanzhulian with higher sesamin or sesamolin contents could be used in functional food, and pharmaceutics or cosmetic industries. Moreover, these cultivars will be potential resources for breeding new cultivars with high sesamin and sesamolin contents and acceptable yield traits.

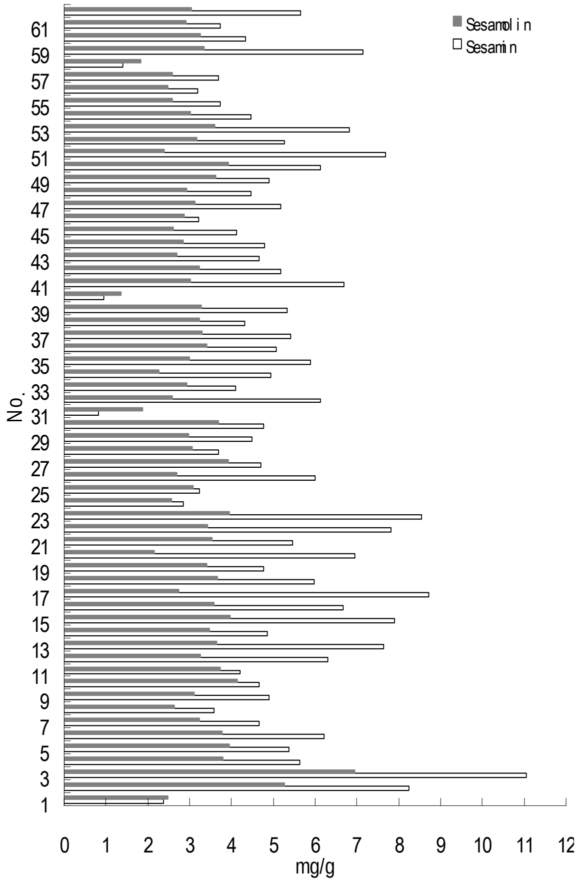

Fig. 3. Variation of the sesamin and sesamolin contents in the 62 cultivars.

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