

Variation in Fatty Acid Composition in Indian Germplasm of Sesame

Nupur Mondal · K. V. Bhat · P. S. Srivastava

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Abstract A germplasm collection of 33 entries comprising 22 sesame (*Sesamum indicum* L.) cultivars, 4 landraces of *S. mulayanum* and 7 other accessions of 4 wild species were analyzed for the fatty acid compositions of their seed oil. The entries varied widely in their fatty acid compositions. The percentage content of oleic, linoleic, palmitic and erucic acids ranged between 36.7–52.4, 30.4–51.6, 9.1–14.8 and 0.0–8.0, respectively. Linolenic and arachidonic acids were the minor constituents but varied widely in wild species. Oleic and linoleic were the major fatty acids with mean values of 45.9 and 40.5%, respectively and the mean of their combined values was 86.4%. The polyunsaturated fatty acid (PUFA) compositions ranged from 30.9 to 52.5% showing high variation in PUFA in the germplasm. Linoleic acid content was very high in one landrace (47.8) and one accession each of three wild species, *S. mulayanum* (49.3), *S. malabaricum* (48.2) and *S. radiatum* (51.6%). Use of fatty acid ratios to estimate the efficiency of biosynthetic pathways resulted in high oleic and low linoleic desaturation ratios and consequently high linoleic and very low linolenic acid contents in seed oil. The results of this study provided useful background information on the germplasm and also identified a few accessions having high linoleic acid which can be used for developing cultivars with desirable fatty acid compositions.

Keywords Sesame · Indian germplasm · Fatty acid composition · Gas chromatography · Poly unsaturated fatty acid · *Sesamum indicum* L.

Introduction

Sesame (*Sesamum indicum* L.), one of the ancient and very important oil seed crops, is widely cultivated in the tropical parts of Africa and Asia. The genus *Sesamum* (family Pedaliaceae) is made up of 36 species besides *S. indicum*, the predominant cultivated species. The origin of sesame is disputed and two possible centers of origin have been proposed: Ethiopia or the Indian subcontinent [1]. Today, India is one of the two largest producers of sesame in the world. The crop is grown across various ecogeographical regions of the country [2].

Sesame seeds and their oil have long been used for human consumption and industrial purposes, such as pharmaceuticals, cosmetics, perfumery, soaps, paints and insecticides. As an oil seed crop, sesame is still very significant world wide [1]. Sesame seeds are mostly used for extracting cooking oil [3]. As a culinary oil, it is flavorful, free of unwanted odors and resistant to oxidative deterioration. The suitability of a vegetable oil for a particular use such as nutritional, industrial or pharmaceutical applications depends upon its fatty acid composition. However, the fatty acid composition of vegetable oil is highly variable in different plant species. In sesame, oleic and linoleic acids are the predominant fatty acids and form more than 80% of the total. The high levels of monounsaturated and polyunsaturated fatty acids (PUFAs) increase the quality of the oil for human consumption. Moreover, high levels of linoleic acid, a PUFA, reduce blood cholesterol and play an important role in preventing atherosclerosis [4]. As the

N. Mondal (✉) · K. V. Bhat
National Research Centre on DNA Fingerprinting,
NBPGR, Pusa Campus, New Delhi 110012, India
e-mail: nupur.mondal84@gmail.com

P. S. Srivastava
Department of Biotechnology, Jamia Hamdard,
Hamdard Nagar, New Delhi 110062, India

demand for beneficial PUFAs has drastically increased in recent years, there are increasing efforts to find plant sources of PUFAs that are economical and sustainable, unlike animal sources. The amount of monounsaturated and PUFAs, in a plant species, depends upon the efficiency with which the process of desaturation and elongation takes place in the biosynthetic pathway. Therefore, desaturation of fatty acids is also an important aspect in oil biochemistry as it determines the level of unsaturation and the economic value of oil [5, 6].

The studies on fatty acid composition of several germplasm collections of crop plants have revealed wide variation, offering possibilities of developing superior quality edible oils and specialized industrial oils [1, 7–10]. Sesame has been grown in India for many years under a range of agronomic conditions. Therefore, considerable variation in oil content and fatty acid composition is expected in the germplasm. Moreover, identification and use of wild relatives with desirable genes has become more relevant today because using biotechnological tools, transfer of genes across species/genera is now feasible. It is, therefore, imperative to survey the fatty acid compositions of the Indian germplasm to identify genotypes for improvement of varieties with superior quality of edible oil.

Limited information is available on the systematic study on the variability pattern in biochemical constituents of sesame germplasm grown in India. Awasthi and Sharma [2] reported fatty acid profiles of 211 promising genotypes while, Hiremath et al. [11] reported fatty acid composition in six wild species of *Sesamum*. However, none of these studies is thorough on fatty acid compositions of germplasm comprising cultivars, landraces and wild species. The present investigation was carried out on sesame germplasm comprising 22 cultivars, 4 landraces and 7 accessions of 4 wild species with the following objectives: (a) to evaluate fatty acid composition of seeds, (b) to compare the desaturation capability and (c) to correlate unsaturated and PUFAs with the desaturation ratios.

Experimental Procedures

Plant Materials Used

The plant materials used included 22 cultivated varieties of *Sesamum indicum*, 4 landraces of *S. mulayanum* and 7 accessions of 4 wild species i.e., *S. mulayanum*, *S. malabaricum*, *S. alatum* and *S. radiatum*. The materials were collected from different states (provinces) of India as given in Table 1. The seeds were collected from the plants grown under irrigated conditions in the field of NBPGR during the month of May to October, 2008 when the average minimum and maximum temperatures recorded in degree Celsius (°C) were 24.2 and 39.0 (May), 27.4 and 41.4 (June),

Table 1 The plant materials used: (A) cultivated varieties of *Sesamum indicum* and (B) land races and wild species of sesame

Cultivars	Pedigree	Place of adaptation
(A) Cultivars of <i>Sesamum indicum</i>		
Punjab Til 1	Selection from local collection	Punjab
Phule Til 1	D-7-11-1 × N-58-2	Maharashtra
JLT 26	Phule Til 1 × N-32	Maharashtra
Thilothama	PT 58-35 × Kayankulam 1	Kerala
Gujarat Til 1	Selection from MT 67–52	Gujarat
Gujarat Til 2	Gujarat Til 1	Gujarat
YLM 11	Vinayak × Kanak	Andhra Pradesh
RT 54	A 65 × BS 61	Rajasthan
RT 103	C 7 × A 65	Rajasthan
Usha	Mutant of variety Kanak	
Shekhar	T 4 × T 12	Uttar Pradesh
AKT 101	N 62 × N 12–19	Maharashtra
TC 25	Punjab Til 1 × EC 20778	Rajasthan
RT 46	T12 × Punjab Til 1	Rajasthan
HT 1	Selection in NP-6–3	HAU, Haryana
Madhavi	Selection from local collection	Andhra Pradesh
Krishna	M 3-2 × Venezualum 17/4	Bihar
T 78	NP-6 × T 4	Uttar Pradesh
CST 2001	Kanpur, UP	Kanpur, UP
Uma	Selection in Kanak	Orissa
DS1 IC 295957	Dhadwad	Dhadwad
AKT 64	N 128 × C 50	Maharashtra
Accessions	Species	Place of collection
(B) Land races and wild species		
Land races		
BB 2601	<i>S. mulayanum</i>	Osian, Jodhpur
BB 2615	<i>S. mulayanum</i>	Kalandri, Sirohi
BB 2609	<i>S. mulayanum</i>	Kharda, Pali
BB 2608	<i>S. mulayanum</i>	Kharda, Pali
Wild form		
CAZRI	<i>S. mulayanum</i>	Rajasthan
ABB-99-33	<i>S. mulayanum</i>	Rajasthan
IC 253971	<i>S. malabaricum</i>	Kayangulam, Kerala
IC 253959	<i>S. malabaricum</i>	Tindivanam
IC 253950	<i>S. alatum</i>	Karur, Tamil Nadu
ABB-99-12	<i>S. alatum</i>	Karur, Tamil Nadu
IC 253968	<i>S. radiatum</i>	Trivandrum

26.6 and 35.8 (July), 26.1 and 34.7 (August), 23.2 and 33.4 (September) and 16.4 and 32.7 (October).

Oil Extraction

One gram of seeds of each genotype was crushed in a mortar and pestle using liquid nitrogen to make a fine

powder. This was followed by extraction of oil for 6 h in a Soxhlet apparatus using petroleum ether.

Fatty Acid Methyl Esterification

A sample of each accession was converted to its fatty acid methyl esters (FAME) according to the method followed by Vasudev et al. [12] with few modifications. Oil extracted from 1 g seed was mixed with 5 mL methanol (AR Grade) and 5 drops of conc. H₂SO₄ (AR Grade, 98% purity). This was then heated in a water bath at 75 °C for 1 h. Then, 2 mL of GC-grade hexane was added. Samples were vigorously shaken for 1 min at room temperature. The mixture was allowed to settle for 25 min and only the upper layer, containing hexane with methyl esters, was taken and used for gas chromatography analysis.

Gas Chromatography

The methyl esters of the fatty acids (1 mL) were analyzed in a Perkin-Elmer Clarus 500 Gas Chromatograph (Perkin-Elmer Waltham, MA, USA) equipped with a flame ionization detector (FID) and a capillary column (30 m long × 0.53 mm diameter). The temperature of both injector and detector was maintained at 250 °C. The carrier gas was nitrogen. The peak identification was performed by comparing the relative retention times with those of a commercial standard mixture of FAME. The fatty acid contents of palmitic (C16:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidonic (C20:4) and erucic (C22:1) were determined using a computing integrator and shown as percentages of the oil. The values presented are the averages of the duplicate fatty acid analyses.

Fatty Acid Ratios

It is difficult to evaluate the potential of different phenotypes for plant breeding by comparing individual fatty acid values because they are intercorrelated and any breeding modification will affect the whole system [13]. For this reason, two ratios were additionally used i.e., oleic desaturation ratio (ODR) and linoleic desaturation ratio (LDR). The ODR and LDR are calculated following Pleines and Friedt [14] and they estimate, within the desaturation pathway, the efficiency of the desaturation from oleic to linoleic (ODR) and from linoleic to linolenic acid (LDR). They were calculated as follows:

$$\text{ODR} = \frac{\% \text{C18:2} + \% \text{C18:3}}{\% \text{C18:1} + \% \text{C18:2} + \% \text{C18:3}}$$

$$\text{LDR} = \frac{\% \text{C18:3}}{\% \text{C18:2} + \% \text{C18:3}}$$

Statistical Analysis

BIOSTAT software was used to find the correlation between individual fatty acids and between UFA, PUFA and desaturation ratios.

Results and Discussion

Fatty Acid Profile

The fatty acid compositions and their range of variation in different accessions are shown in Table 2. Oleic and linoleic acids were found to be the major fatty acids in the germplasm investigated. The percentage of oleic acid in seed oil ranged from 36.7 to 52.4% with a mean value of 45.9%. Linoleic acid varied between 30.4 and 51.6% with a mean value of 40.5% (Table 2). Thus, oleic acid content was found to be higher than that of linoleic acid. Similar results of oleic and linoleic acid contents (43.2 and 40.0%, respectively) and also of oleic/linoleic acid ratio were reported when 211 entries of Indian sesame lines, collected under All India Coordinated Research Project (AICRP), were analyzed [2]. Earlier, it was reported that the composition of fatty acids in sesame as well as the oleic/linoleic acid ratios are greatly affected by environment [15]. However, for the present study, all the 33 entries were grown in the same year at the same place before collecting the seeds for analysis. In contrast, seeds of the 211 entries for the study by other group [2], were collected from various AICRP centers situated at different ecogeographical regions of the country. Despite this, the display of similar results by the two collections of Indian sesame germplasm indicates that the results were mostly influenced by genotype rather than environmental conditions of the locations where the materials were grown. Again, in the present study, the means of oleic and linoleic acids as unsaturated fatty acids, was 86.4%. This mean value is similar to that of the world collection (85.6%), the exotic introduction in Sudan (86.1%) and Sudanese cultivars (85.6%) but higher than that of the Saudi Arabian collection (79.3%) [16–18]. Thus, the materials of the present study had higher contents of these two fatty acids in comparison with those of the Saudi Arabian collections. One possible reason for this, as suggested by Uzun et al. [1], might be that the materials of the present study were a heterogeneous collection and originally obtained from various states of India (Table 1) suited to different environmental conditions.

The higher mean values of the two unsaturated fatty acids of sesame, recorded during this study, are significant because oleic and linoleic acids increase the oil quality for human consumption. The highest amount of oleic acid was

Table 2 Range of variation in fatty acid composition (% in oil) of sesame germplasm

Genotype	Palmitic	Oleic	Linoleic	Linolenic	Arachidonic	Erucic
Phule Til 1	12.27	47.44	39.10	0.61	0.18	0.40
JLT 26	13.38	46.97	35.40	0.33	0.99	2.93
Thilothama	9.06	46.86	42.25	0.58	0.82	0.43
PB Til 1	10.50	44.04	43.83	0.62	0.72	0.29
Guj Til 1	11.53	45.36	42.32	0.41	0.07	0.31
Guj Til 2	10.94	43.95	43.41	0.50	0.78	0.42
YLM 11	11.03	46.67	40.37	0.48	0.91	0.54
RT 54	10.64	45.26	42.35	0.50	0.88	0.37
RT 103	11.42	48.69	38.42	0.50	0.06	0.91
Usha	11.24	44.37	42.67	0.46	0.86	0.40
Shekhar	10.49	45.08	42.96	0.43	0.80	0.24
AKT 101	14.45	48.16	31.54	1.17	1.06	3.62
TC 25	10.79	49.47	38.06	0.40	0.84	0.44
RT 46	11.30	49.68	33.92	0.27	0.39	4.44
HT1 DF 105	11.08	42.54	44.47	0.38	1.17	0.36
Madhavi	10.30	47.55	41.54	0.33	0.05	0.23
Krishna	10.27	45.51	42.59	0.47	0.89	0.27
DS1 IC 295957	10.78	48.67	38.81	0.41	0.91	0.42
CST	11.05	47.95	40.00	0.52	0.21	0.27
Uma	11.93	46.60	39.89	0.29	0.12	1.17
T78	14.79	50.36	30.38	0.27	0.22	3.98
AKT 64	12.13	46.24	40.85	0.50	0.09	0.19
BB 2601	9.60	40.89	47.76	0.55	0.90	0.30
BB 2615	10.81	46.36	31.39	0.50	2.94	8.00
BB 2609	9.96	49.13	38.34	0.88	1.39	0.30
BB 2608	9.61	42.73	44.44	0.89	0.95	1.38
<i>S. mulayanum</i> CAZRI	11.49	52.39	31.80	0	1.02	3.30
<i>S. mulayanum</i> ABB-99-33	9.68	40.08	49.25	0	0	0.99
<i>S. malabaricum</i> IC 253971	11.87	49.60	34.59	0	3.00	0.94
<i>S. malabaricum</i> IC 253959	9.88	40.19	48.15	0.40	1.01	0.37
<i>S. alatum</i> IC 253950	10.32	44.83	44.85	0	0	0
<i>S. alatum</i> ABB-99-12	11.26	45.54	39.55	0.20	0	3.45
<i>S. radiatum</i> IC 253968	10.52	36.66	51.64	0.42	0.05	0.71

found to be present in T 78 (50.4%), BB 2609 (49.1) and *S. mulayanum* CAZRI (52.4) among cultivars, landraces and wild accessions, respectively. Linoleic acid was found to be highest in HT1 DF 105 (44.5), among the cultivars. However, very high linoleic acid was recorded in one landrace, BB 2601 (47.8) and one accession each of three wild species like *S. mulayanum* ABB-99-33 (49.3), *S. malabaricum* IC 253959 (48.2) and *S. radiatum* IC 253968 (51.6) making them nutritionally valuable. Since edible oil with a high linoleic acid content is a premium oil, these accessions having high linoleic acid content could be used for improving the linoleic acid content in sesame cultivars through breeding. There were few entries like Guj Til 2, PB Til 1 and *S. alatum* IC 253950 in which the oleic and linoleic acid contents were in proportion of almost 1:1

and the total of these two unsaturated fatty acids were more than 87%. Therefore, these entries are considered to be important sources for improving new varieties with both high oleic and linoleic acid content as pointed out by Uzun et al. [1].

Considering the beneficial health effects of sesame oil, there is a need to develop varieties with a high linolenic acid content. However, fatty acids are formed by a stepwise biosynthetic pathway in which oleic acid undergoes desaturation to form linoleic and then linolenic acid. Therefore, the main focus of this study was on oleic, linoleic and linolenic acids so as to use the data in a continuous ongoing study to find genes responsible for the alpha-linolenic acid (ALA) production in sesame. Thus, the estimation of saturated fatty acids received a lower priority.

Stearic acid was not estimated or included in tables or calculations. However, palmitic acid, the predominant saturated fatty acid of sesame oil was estimated and found to range from 9.1 to 14.8% and have a mean value of 11.1%.

Although linolenic acid was one of the most important PUFAs in this study, its quantity was very small, ranging from 0.0 to 1.2 (highest in AKT-101) with a mean value of 0.4%. The results were in agreement with those of earlier reports on sesame showing either no [17] or about 0.4% linolenic acid [19]. Amongst all the studied germplasm, the landraces of *S. mulayanum* had a higher quantity of linolenic acid (0.7%) as compared to its wild counterparts (0.0%). Also, cultivated varieties of *S. indicum* were found to have a higher quantity of linolenic acid (0.5%) compared to wild species investigated (0.2%). Arachidonic acid, another PUFA, was also found to be a minor constituent in sesame with a mean value of 0.8% but with large variation in wild accessions (0.0–3.0%). Similarly, erucic acid, the other monounsaturated fatty acid estimated, was also low but with very large variation ranging from 0.0 to 8.0% with a mean value of 1.3% (Table 2).

Unsaturated Fatty Acids and Desaturation Ratios

Variations in unsaturated (UFA) and PUFAs are shown in Table 3. Considering all the fatty acids, the average of unsaturated fatty acids was 88.9%. The highest UFA was found in a cultivar of *S. indicum*, Thillothama (90.9%). The high level of UFA increases the quality of the oil for human consumption. The PUFAs were found to be highest in HT1 DF 105 (46.0), BB 2601 (49.2) and *S. radiatum* IC 253968 (52.5%) among cultivars, landraces and wild species, respectively. The high level of PUFA also helps in increasing the health quality of oil especially in combating heart disease. Oils containing 50% or more PUFAs have not only health benefits but also several industrial applications especially in the manufacture of oil based paints [20].

The fatty acid ratios in this evaluation were very useful to estimate the relative efficiency of the desaturation pathways, and their use will help design strategies for future breeding programs involving the present germplasm. The variability in the values of ODR and LDR i.e., the efficiency of the desaturation systems from C18:1 to C18:2 and from C18:2 to C18:3, respectively, are shown in Table 3. Mean value of ODR (0.5) was found to be quite high in comparison with that of LDR (0.01). These values explain the large increase of C18:2 and decrease in C18:3. The value of ODR was highest in *S. radiatum*, acc. no. IC 253968 (0.6). The high value of ODR represented a considerably high amount of linoleic acid (Table 2). In the germplasm studied, LDR showed a very low amount, indicating low levels of linolenic acid formation in sesame. The highest value of LDR can be observed in AKT

Table 3 Range of variation in unsaturated, polyunsaturated fatty acids and desaturation ratios in oil of sesame germplasm

Genotype	UFA	PUFA	ODR	LDR
Phule Til 1	87.73	39.89	0.45	0.015
JLT 26	86.62	36.72	0.43	0.009
Thillothama	90.94	43.65	0.47	0.013
PB Til 1	89.5	45.17	0.50	0.014
Guj Til 1	88.47	42.80	0.48	0.009
Guj Til 2	89.06	44.69	0.50	0.011
YLM 11	88.97	41.76	0.46	0.011
RT 54	89.36	43.73	0.48	0.011
RT 103	88.58	38.98	0.44	0.012
Usha	88.76	43.99	0.49	0.01
Shekhar	89.51	44.19	0.49	0.009
AKT 101	85.55	33.77	0.40	0.036
TC 25	89.21	39.30	0.44	0.011
RT 46	88.70	34.58	0.41	0.007
HT1 DF 105	88.92	46.02	0.51	0.008
Madhavi	89.70	41.92	0.46	0.007
Krishna	89.73	43.95	0.48	0.011
DS1 IC 295957	85.21	40.13	0.44	0.010
CST	88.95	40.73	0.46	0.012
Uma	88.07	39.30	0.46	0.007
T 78	89.22	30.85	0.38	0.008
AKT 64	87.87	41.54	0.47	0.014
BB 2601	90.40	49.21	0.54	0.011
BB 2615	89.19	34.30	0.40	0
BB 2609	90.04	40.61	0.44	0.022
BB 2608	90.39	46.28	0.51	0.019
<i>S. mulayanum</i> CAZRI	88.51	32.82	0.37	0
<i>S. mulayanum</i> ABB-99-33	90.32	49.25	0.55	0
<i>S. malabaricum</i> IC 253971	88.13	37.59	0.41	0
<i>S. malabaricum</i> IC 253959	90.12	49.56	0.54	0.008
<i>S. alatum</i> IC 253950	89.68	44.83	0.50	0
<i>S. alatum</i> ABB-99-12	88.74	40.54	0.47	0.024
<i>S. radiatum</i> IC 253968	89.48	52.49	0.58	0.015

UFA unsaturated fatty acid, PUFA polyunsaturated fatty acid, ODR oleic desaturation ratio, LDR linoleic desaturation ratio

101 (0.04) and this corresponds to its high linolenic acid content (Table 2). Relatively higher average values of ODR and LDR explain the increase of C18:3 content [7]. The high ODR values imply that the biosynthetic pathway of fatty acids is efficient in the formation of oleic to linoleic acids. Thus, oleic and linoleic acids are the major constituents of sesame oil.

Association of Individual Fatty Acids

The results of correlation analyses among fatty acids of the germplasm examined are shown in Table 4. A significant

Table 4 Relationships among fatty acid compositions (% in oil) in sesame germplasm

	PALMITIC	OLEIC	LINOLEIC	LINOLENIC	ARACHIDONIC	ERUCIC
Pearson Correlation Matrix						
PALMITIC	1.000					
OLEIC	0.578*	1.000				
LINOLEIC	-0.545*	-0.692*	1.000			
LINOLENIC	0.166	-0.022	0.172	1.000		
ARACHIDONIC	-0.419*	-0.387*	-0.257	-0.333*	1.000	
ERUCIC	-0.027	-0.132	-0.585*	-0.311*	0.716*	1.000

* Significant at 95.0% confidence interval

Table 5 Relationships between unsaturated, polyunsaturated fatty acids and desaturation ratios in oil of sesame germplasm

	UFA	PUFA	ODR	LDR
Pearson Correlation Matrix				
UFA	1.000			
PUFA	0.536*	1.000		
ODR	0.501*	0.982*	1.000	
LDR	-0.318 *	-0.043	-0.018	1.000

UFA unsaturated fatty acid PUFA polyunsaturated fatty acid, ODR oleic desaturation ratio, LDR linoleic desaturation ratio

* Significant at 95.0% confidence interval

positive correlation was observed between oleic and palmitic acid, a desirable and an undesirable fatty acid, respectively. This result is in agreement with previous reports on sesame [1] and opium poppy [10]. Erucic acid also showed a strong positive correlation with arachidonic acid but a negative correlation with both linoleic and linolenic acids. Oleic acid showed a very strong negative correlation with linoleic acid. This association was well documented in sesame earlier [1, 16, 21]. Correlations between oleic and linoleic acids were always found to be strongly negative in many oil seed crops [1]. However, in the present study, significant negative correlations were also recorded between palmitic and linoleic, palmitic and arachidonic and, between erucic and linoleic acids (Table 4).

Association of Unsaturated Fatty Acids and Desaturation Ratios

The relationship between unsaturated and PUFAs in sesame oil and the desaturation ratios are shown in Table 5. ODR was found to have a significantly negative correlation with LDR. PUFA was found to have a significant positive correlation with ODR. This was found to be true as linoleic acid constitutes a major fraction of the total fatty acids and hence, PUFA, of sesame.

The germplasm in this study was found to have considerable variability in fatty acid composition. The large inter- and intra-specific variability observed offers interesting prospects. The major fatty acids in the oil, on average, were oleic and linoleic acids. One landrace and one accession each of three wild species could be identified as having very high linoleic acid and consequently, higher PUFA, thus nutritionally valuable. Therefore, results obtained in this study provide useful background information for developing new cultivars with appropriate fatty acid composition which would be beneficial for combating human health problems.

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