Fatty Acid Compositions in Newly Differentiated Tissues of Soybean Seedlings

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Six soybean genotypes with various fatty acid compositions were allowed to germinate in sand under a greenhouse. Seedlings were picked up after 5, 6, 7, 8, 10, and 12 days and dried under sun. During germination, the fatty acid composition of cotyledons changed slightly, but those of all newly differentiated tissues, including root, hypocotyl, epicotyl, unifoliate, and trifoliate, changed dramatically, especially within the first 5 days. Although lipids from these tissues contained the five major fatty acids found in original seeds, their fatty acid compositions were totally different from that of the seed: higher in palmitic, linolenic, and stearic acid and lower in oleic and linoleic acid. There existed a distinct difference in fatty acid compositions between the young tissues below and those above cotyledons in the seedling. Furthermore, all new tissues conserved their fatty acid compositions regardless of genotypes, which may be explained by conservation of membrane lipids predominating in these tissues.

Keywords: *Soybeans; germination; fatty acid composition; young tissues; lipids*

INTRODUCTION

Soybean oil is an increasingly valuable commodity in world trade, with global production in the region of 17.7 million metric tons between 1993 and 1994 (*1994 Soya Bluebook*, 1994). However, the fatty acid composition of soybean oil is often not considered ideal in terms of oil functionality and oxidative stability. Although industrial hydrogenation is as effective as plant breeding in altering the fatty acid composition of oils, its byproduct of *trans* fatty acids has been shown to have possible adverse health implications (Mensink and Katan, 1990). As a result, plant breeding is increasingly becoming a priority approach in modifying the fatty acid composition of soybean oil, particularly when it is combined with rapidly emerging biotechnology.

If the fatty acid composition of soybean oil is to be altered by genetic manipulation, it is necessary to know how the fatty acids in the soybean plant are metabolized. It is known that the soybean plant stores oil in its seeds in the form of triglycerides. These storage reserves are synthesized during seed development and then used as carbon and energy sources during seed germination (Murphy, 1990). Although previous investigators studied changes in fatty acid composition during soybean seed development (Howell and Collins, 1957; Rubel et al., 1972) as well as germination (Brown et al., 1962; Singh et al., 1968), little is known of the changes of fatty acid compositions in newly differentiated tissues of soybean seedlings (Joshi et al., 1973). The objective of this study was to provide additional information on these aspects.

MATERIALS AND METHODS

Germination. Soybean seeds of six selected genotypes with various fatty acid compositions were allowed to germinate in sand under greenhouse conditions. After 5, 6, 7, 8, 10, and 12 days of germination, seedlings were picked up and dried under sunlight. Cotyledons and newly differentiated tissues were then separated from individual seedlings by a hand knife.

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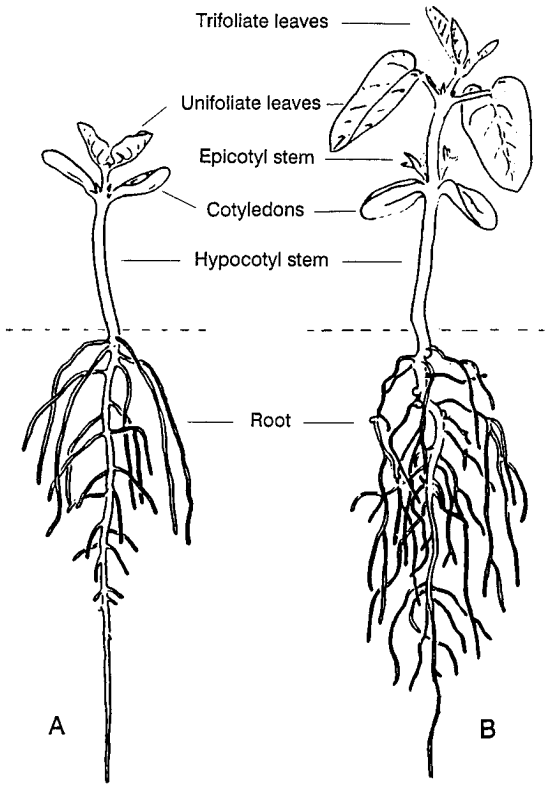

Figure 1. Schematic diagram of soybean seedlings showing newly differentiated tissues at two germination stages: (A) after 5 days; (B) after 12 days.

As shown in Figure 1, these new tissues included root, hypocotyl stem, epicotyl stem, unifoliate leaves, and trifoliate leaves. Tissue samples of the same type from different seedlings were finally combined before being analyzed for the relative percentage of fatty acid composition.

Analysis of Fatty Acid Composition. The whole seed sample as well as the combined dry samples of each seedling tissue from each of six soybean genotypes were ground with a coffee mill. For whole seeds and the cotyledon tissue, 20 mg of dry sample was used for derivatizing their lipids into fatty acid methyl esters (FAMEs), whereas for other tissues, 40 mg of dry sample were needed. FAMEs were prepared by direct transmethylation without extraction of lipids from the tissues,

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Table 1. Fatty Acid Composition in Seeds of Six Selected Soybean Genotypes before Germination[a]

genotype	fatty acid composition (rel %)				
	C16:0	C18:0	C17:1	C18:2	C18:3
A	12.1	4.4	17.5	55.8	9.1
B	11.8	4.1	24.3	51.2	7.8
C	11.6	4.7	26.0	50.3	6.8
D	9.8	21.7	17.6	42.8	5.6
E	12.2	4.3	23.4	55.5	3.4
F	4.6	3.1	37.4	48.7	5.3

[a] Means of duplicate measurements.

Table 2. Changes in Fatty Acid Composition of Soybean Cotyledons during Germination[a]

genotype	days of germination	fatty acid composition (rel %)				
		C16:0	C18:0	C18:1	C18:2	C18:3
A	0	12.4	4.4	17.3	55.0	9.9
	5	13.4	6.0	12.4	55.9	11.1
	6	16.3	6.2	9.8	52.9	12.8
	7	14.8	5.4	14.0	49.5	12.3
	12	18.8	7.4	14.5	46.8	12.5
B	0	11.9	4.2	23.8	51.8	7.6
	5	12.4	4.2	18.0	54.4	8.9
	6	14.0	5.0	14.0	54.0	12.0
	7	14.8	5.4	14.0	49.5	12.3
	12	21.4	6.5	16.8	42.8	12.4
C	0	11.5	5.1	26.2	50.5	6.4
	5	14.1	5.0	26.6	46.4	5.8
	6	17.3	6.4	16.7	43.9	12.2
	7	27.1	6.8	9.7	36.3	18.2
	12	26.5	7.7	20.7	35.4	6.7
D	0	9.3	21.5	16.9	44.3	5.5
	5	10.6	22.2	15.2	43.4	6.4
	6	10.5	22.3	12.3	43.5	9.2
	7	13.3	16.8	10.6	45.5	10.6
	12	16.6	22.3	14.3	30.9	12.0
E	0	12.4	4.1	23.2	55.8	3.4
	5	13.3	4.6	18.0	57.5	5.7
	6	11.8	4.4	14.9	57.8	9.3
	7	11.7	4.9	16.9	55.1	9.2
	12	12.6	4.7	23.1	50.6	5.6
F	0	4.8	3.0	37.6	48.0	5.4
	5	6.3	3.0	34.9	47.9	6.0
	6	5.6	3.1	41.2	42.0	6.3
	7	8.3	3.7	33.2	43.6	7.9
	12	13.5	4.6	32.5	39.5	6.9

[a] Means of duplicate measurements.

according to a procedure described by Liu et al. (1995a). For FAME analysis, a gas−liquid chromatographic system fitted with an automatic sampler and flame ionization detector was employed. The detailed running conditions were given elsewhere (Liu et al., 1995b).

RESULTS AND DISCUSSION

Fatty Acid Composition of Six Selected Genotypes. There was a large variation in fatty acid composition of lipids in seeds of six selected soybean genotypes (Table 1). Genotypes A−C had normal fatty acid compositions: palmitic acid (C16:0), 11.6−12.1%; stearic acid (C18:0), 4−4.7%; oleic acid (C18:1), 17.5−26.0%; linoleic acid (C18:2), 50.3−55.8%; linolenic acid (C18:3), 6.8−9.1%. Genotype D was a high stearate line (21.7%). Genotype E was a low linolenic acid line (3.4%), whereas genotype F was a low saturated line (7.7% total).

Changes in Fatty Acid Composition of Cotyledons during Germination. Before germination, the fatty acid composition of soybean cotyledons (Table 2, 0 days of gemination) did not significantly differ from that of whole seeds (Table 1). This finding was reported earlier (Liu et al., 1995a). The explanation was that soybean cotyledons represent about 98% of total lipids present in the whole seed. As seeds germinated and grew, there was a slight but significant ($p < 0.05$) change in fatty acid composition. In general, palmitic and linolenic acid increased, whereas linoleic acid decreased. Oleic acid decreased also up to 7 days of gemination but then increased at 12 days of germination. Change in stearic acid was irregular.

Table 3. Changes in Fatty Acid Composition of the Newly Differentiated Root Tissue during Earlier Stages of Soybean Growth[a]

genotype	days of germination	fatty acid composition (rel %)				
		C16:0	C18:0	C18:1	C18:2	C18:3
A	5	46.7	7.3	6.8	21.3	15.8
	6	54.1	8.0	7.1	17.3	11.0
	7	55.5	9.3	6.7	17.9	10.6
	10	59.1	9.8	3.5	15.3	7.8
	12	48.2	10.0	8.7	18.9	9.2
B	5	49.7	7.3	6.7	21.2	15.0
	6	53.4	7.7	6.3	18.2	12.0
	7	57.2	9.1	6.4	17.2	10.1
	10	53.3	8.7	5.1	14.4	8.2
	12	47.0	8.2	6.0	18.2	11.3
C	5	50.0	8.1	7.8	21.3	12.7
	6	57.0	8.6	6.2	18.2	10.1
	7	53.8	8.7	6.4	19.0	12.0
	10	51.9	8.9	6.3	19.5	11.9
	12	44.5	9.8	6.5	22.0	11.1
D	5	48.8	9.8	6.8	20.0	14.6
	6	50.0	9.9	6.9	20.4	12.8
	7	54.1	10.6	5.9	18.5	10.8
	10	46.8	11.3	5.5	19.9	10.2
	12	43.2	11.3	6.4	20.9	10.1
E	5	50.2	7.1	5.8	24.0	12.8
	6	53.7	7.8	6.3	18.7	8.8
	7	56.9	9.1	5.5	19.6	8.8
	10	55.6	9.1	5.7	20.6	8.9
	12	48.0	7.3	5.2	22.6	8.8
F	5	40.8	9.0	6.7	28.5	13.5
	6	42.3	9.7	8.6	25.2	14.1
	7	45.3	11.1	5.9	17.3	9.7
	10	49.7	11.9	6.2	15.6	8.4
	12	45.4	11.6	6.9	25.9	10.2

[a] Means of duplicate measurements.

There has been a controversy regarding change in fatty acid composition of soybean cotyledons during germination. Brown et al. (1962) reported slight changes of fatty acids in soybean cotyledons of Chippewa variety during germination in the dark up to 12 days. Compared to other fatty acids, oleic acid was found to decrease significantly. Later, Joshi et al. (1973) observed a decrease in palmitic and oleic acids in soybean cotyledons of Lee variety during germination in the dark from 6 to 12 days. However, Singh et al. (1968) found practically no significant changes in fatty acid compositions of soybean seedlings of three varieties during germination in the greenhouse up to 6 days. The observed differences among the studies, including the present one, might be due to differences in germination conditions, germination stage, variety, and analytical techniques employed.

The fact that fatty acid composition in cotyledons changes slightly or little indicates that storage lipids in cotyledons may be broken down and utilized on the basis of individual triglycerides rather than fatty acids.

Table 4. Changes in Fatty Acid Composition of the Newly Differentiated Hypocotyl Stem Tissue during Earlier Stages of Soybean Growth[a]

genotype	days of germination	fatty acid composition (rel %)				
		C16:0	C18:0	C18:1	C18:2	C18:3
A	5	52.1	11.1	3.5	24.0	11.1
	6	50.0	10.3	1.5	26.1	12.4
	7	47.0	11.5	2.1	23.1	12.9
	8	44.1	9.0	1.4	27.9	17.5
	12	42.8	9.8	1.9	30.1	15.2
B	5	50.2	11.4	5.2	22.5	10.6
	6	40.4	10.2	8.5	25.4	10.7
	7	47.0	11.5	2.1	23.1	12.9
	8	39.2	8.5	1.9	27.5	18.6
	12	41.2	9.4	1.9	28.1	18.5
C	5	54.4	12.2	5.0	17.6	6.5
	6	45.4	11.1	4.7	28.1	10.8
	7	42.3	4.6	2.0	28.0	15.5
	8	38.1	9.5	2.9	27.6	16.7
	12	39.2	9.8	3.9	24.0	17.1
D	5	40.2	25.4	4.3	19.0	6.4
	6	40.0	19.4	4.3	25.6	10.7
	7	34.2	17.9	2.4	27.1	13.6
	8	28.2	15.1	6.1	34.8	15.7
	12	35.1	14.5	3.9	31.0	15.4
E	5	55.0	11.9	2.5	21.3	9.8
	6	48.0	12.1	2.1	23.9	13.4
	7	43.8	10.9	1.9	27.0	15.7
	8	36.6	8.7	1.9	32.1	21.2
	12	37.6	8.8	4.7	27.4	18.3
F	5	45.0	13.1	5.6	31.2	4.7
	6	39.0	11.3	4.4	28.9	9.7
	7	33.9	9.8	3.0	32.0	13.2
	8	37.2	10.1	3.8	32.3	13.5
	12	37.9	12.6	4.6	33.0	11.8

[a] Means of duplicate measurements.

Fatty Acid Composition in the Newly Differentiated Root and Hypocotyl Stem. As measured on the fifth day of germination, lipids in the newly differentiated root and hypocotyl stem tissues of six genotypes consisted of all five major fatty acids found in the original seed. However, their fatty acid compositions (Tables 3 and 4) differed significantly from that of the original seed (Table 1). Instead, the newly differentiated tissues had their unique fatty acid composition regardless of genotypes: highest proportion of C16:0, followed by C18:2 and then C18:3, and lowest in C18:1. As days of germination increased from 5 to 12, all fatty acid components in the hypocotyl stem changed (Table 4), with C16:0, C18:0, and C18:1 decreasing and C18:2 and C18:3 increasing. In roots, noticeable changes were also observed (Table 3), especially at the earlier stage of germination, with C18:3 decreasing, C18:0 increasing, C16:0 increasing first and then decreasing, and C18:2 decreasing first and then increasing.

Fatty Acid Composition in the Epicotyl Stem, Unifoliate and Trifoliate Leaves. The fatty acid compositions in the newly differentiated epicotyl stem (Table 5), unifoliate leaves (Table 6), and trifoliate leaves (Table 7) were very similar to each other among all genotypes. They were all characterized by higher proportions of C16:0, C18:3, and as well as C18:2 and the lowest proportion of C18:1. These compositions were again dramatically different from that of the original seed (Table 1). Furthermore, among the five growing tissues, those below and those above cotyledons showed some distinction in terms of their fatty acid compositions; those below cotyledons (root and hypocotyl stem) had higher palmitic acid but lower linolenic acid than those above cotyledons.

Table 5. Changes in Fatty Acid Composition of the Newly Differentiated Epicotyl Stem Tissue during Earlier Stages of Soybean Growth[a]

genotype	days of germination	fatty acid composition (rel %)				
		C16:0	C18:0	C18:1	C18:2	C18:3
A	8	32.1	11.4	1.3	29.9	26.1
	10	35.9	10.0	1.5	31.1	21.1
	12	38.4	11.1	2.1	29.4	18.4
B	8	32.1	9.3	1.9	26.1	30.8
	10	36.9	9.5	2.1	27.1	25.1
	12	35.1	9.3	3.2	26.7	25.7
C	8	32.3	11.4	2.7	22.9	24.8
	10	36.9	10.8	2.6	22.4	21.7
	12	42.0	12.3	3.7	23.9	18.1
D	8	34.1	12.2	1.5	25.7	24.0
	10	34.6	12.8	2.0	26.6	21.3
	12	37.2	12.0	4.2	25.3	19.0
E	8	34.3	10.4	1.5	25.6	25.3
	10	35.6	11.8	2.3	24.5	21.6
	12	37.2	9.7	2.3	28.1	21.2
F	8	33.1	16.2	2.1	26.9	22.1
	10	35.0	16.1	2.0	27.2	20.1
	12	33.7	13.6	3.5	28.0	21.2

[a] Means of duplicate measurements.

Table 6. Changes in Fatty Acid Composition of the Newly Differentiated Unifoliate Leaves during Earlier Stages of Soybean Growth[a]

genotype	days of germination	fatty acid composition(rel %)				
		C16:0	C18:0	C18:1	C18:2	C18:3
A	5	42.5	9.4	2.1	19.6	23.6
	6	36.7	9.8	2.2	15.9	28.6
	7	40.6	10.2	2.5	17.1	24.5
	8	42.8	8.3	3.1	12.5	24.3
	12	35.9	7.1	4.1	10.2	35.4
B	5	41.8	11.0	3.6	19.5	17.1
	6	36.7	9.8	2.2	15.9	28.6
	7	38.1	8.4	2.3	14.6	30.8
	8	40.2	7.6	2.7	11.5	29.4
	12	35.9	7.1	4.1	10.2	35.4
C	5	43.9	12.7	3.2	16.6	17.9
	6	40.9	10.6	2.3	16.6	23.7
	7	37.3	8.2	2.0	11.0	30.7
	8	36.8	6.8	2.5	11.0	33.6
	12	33.6	6.9	1.9	9.6	34.1
D	5	40.6	13.8	4.0	20.8	17.2
	6	39.3	11.1	2.9	18.8	24.8
	7	38.8	9.0	3.3	15.8	28.2
	8	39.6	8.3	3.0	12.1	26.7
	12	36.1	6.9	2.9	8.4	33.3
E	5	46.5	11.0	3.9	21.7	13.3
	6	38.0	10.2	2.6	20.3	22.0
	7	34.2	8.2	2.2	17.6	29.2
	8	39.1	8.0	2.7	11.9	30.1
	12	33.2	7.8	2.7	11.2	34.6
F	5	33.6	11.5	7.3	26.5	11.3
	6	33.4	11.3	7.4	24.4	19.3
	7	31.3	9.7	3.0	16.7	24.9
	8	33.4	8.6	2.9	15.9	28.1
	12	31.0	6.8	3.6	12.5	38.1

[a] Means of duplicate measurements.

Although there were significant changes in fatty acid compositions of the tissues above cotyledons during 5−12 days of gemination, the changes were only minor. In general, in the epicotyl tissue (Table 5), C16:0 and C18:1 increased while C18:3 decreased. C18:0 and

Table 7. Changes in Fatty Acid Composition of the Newly Differentiated Trifoliate Leaves during Earlier Stages of Soybean Growth[a]

genotype	days of germination	fatty acid composition (rel %)				
		C16:0	C18:0	C18:1	C18:2	C18:3
A	10	38.0	11.0	1.7	21.1	18.6
	12	32.5	8.6	2.0	18.9	30.8
B	10	35.0	9.4	1.4	25.4	20.5
	12	32.5	8.6	2.0	18.9	30.8
C	10	36.2	10.6	2.0	21.3	27.4
	12	28.7	6.8	1.3	15.1	36.2
D	10	37.6	11.6	1.9	23.9	20.1
	12	31.9	10.3	1.8	19.0	33.7
E	10	34.3	9.7	1.5	25.3	21.8
	12	35.0	9.5	1.5	23.5	28.4
F	10	31.4	10.9	1.5	28.4	16.9
	12	26.8	8.8	2.0	26.3	22.4

[a] Means of duplicate measurements.

C18:2 changed little. However, in both unifoliate and trifoliate leaves (Tables 6 and 7), the changes were almost opposite: C16:0, C18:0, and C18:2 decreased; C18:3 increased; C18:1 increased in some genotypes but decreased in others. Again, these newly differentiated tissues had their unique fatty acid compositions regardless of genotype. In other words, the fatty acid compositions in all new tissues are conserved within soybean species.

No previous studies have been reported on changes in fatty acid composition of the newly differentiated tissues in soybeans except for the root tissue. Joshi et al. (1973) found that the major fatty acid in roots was also linoleic, but in comparison with cotyledons palmitic acid and linolenic acid were higher. They also reported no major changes in fatty acid composition in the root during 3−12 days of germination. However, our results did not agree with theirs. We found that in lipids of all growing tissues (including roots) palmitic acid was the highest, whereas oleic acid was the lowest. Compared with original seeds, these growing tissues also contained higher percentages of linolenic and stearic acid. The unique compositions of fatty acids in growing tissues may have physiological roles. For example, oleic acid is known to be a central metabolite in plant lipid metabolism (Murphy, 1990). The lowest oleic acid content in the newly differentiated tissues is consistent with the fact that these tissues are physiologically very active during germination.

In an oilseed plant such as the soybean plant, there are two types of lipids: membrane lipids and storage lipids. Storage lipids are mainly triglycerides. They are high in quantity and localized in seed oil bodies of cotyledon tissues. Membrane lipids, however, are mainly phospholipids. They are low in quantity and located in all cells of living tissues. During germination, storage lipids are metabolized through lipolysis, β-oxidation, and glyoxylate cycle and finally converted to sucrose. Sucrose is then transported to the newly differentiated tissues, where it is used as a carbon and energy source for the synthesis of many new biological materials, including proteins, enzymes, and membrane lipids (Murphy, 1990).

In the cotyledon tissue (or the whole seed), the fatty acid composition represents that of storage lipids, whereas in growing tissues that contain almost zero amount of storage lipids, the fatty acid composition represents that of membrane lipids. Since membrane lipids are involved in such fundamental cell processes as ion transport, energy generation, and biological reactions, they are highly conserved in terms of both quantity and quality (Slabas et al., 1995). This may explain why the fatty acid composition in newly differentiated tissues was conserved regardless of genotype.

In conclusion, lipids in all newly formed tissues of soybean seedlings consist of five major fatty acids found in the original seeds. They are C16:0, C18:0, C18:1, C18:2, and C18:3. During gemination, the fatty acid composition in the cotyledon tissue changed slightly, but the fatty acid composition of newly differentiated tissues was significantly different from that of the original seed: higher in palmitic, linolenic, and stearic acid and lower in oleic and linoleic acid. Furthermore, the fatty acid composition of each new tissue, including roots, stems, and leaves, is highly conserved regardless of genotypic variation in original seeds.

LITERATURE CITED

Brown, B. E.; Meade, E. M.; Butterfield, J. R. The effect of germination upon the fat of the soybean. *J. Am. Oil Chem. Soc.* **1962**, *39*, 327−330.

Howell, R. W.; Collins, F. I. Factors affecting linolenic and linoleic acid content of soybean oil. *Agron. J.* **1957**, *49*, 593−597.

Joshi, A. C.; Chopra, B. K.; Collins, L. C.; Doctor, V. M. Distribution of fatty acids during germination of soybean seeds. *J. Am. Oil Chem. Soc.* **1973**, *50* (8), 282−283.

Liu, K. S.; Brown, E. A.; Orthoefer, F. Fatty acid composition within each structural part and section of a soybean seed. *J. Agric. Food Chem.* **1995a**, *43*, 381−383.

Liu, K. S.; Orthoefer, F.; Brown, E. A. Association of seed size with genotypic variation in the chemical constituents of soybeans. *J. Am. Oil Chem. Soc.* **1995b**, *72*, 189−191.

Mensink, R. P.; Katan, M. B. Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N. Engl. J. Med.* **1990**, *323*, 439−445.

Murphy, D. J. Storage lipid bodies in plants and other organisms. *Prog. Lipid Res.* **1990**, *29* (4), 299−324.

Rubel, A.; Rinne, R. W.; Canvin, D. T. Protein, oil, and fatty acid in developing soybean seeds. *Crop Sci.* **1972**, *12*, 739−741.

Singh, B. B.; Hadley, H. H.; Collins, F. I. Distribution of fatty acids in germinating soybean seed. *Crop Sci.* **1968**, *8*, 171−173.

Slabas, A. R.; Simon, J. W.; Elborough, K. M. Information needed to create new oil crops. *Int. News Fats, Oils Related Mater.* **1995**, *6* (2), 159−166.

1994 Soya Bluebook; Soyatech Inc.: Bar Harbor, ME, 1994.

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