Increasing of the Sunflower Genetic Diversity by Mutagenesis

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Abstract

Mutagenic treatments were performed on sunflower cultivars, lines and hybrid seeds with gamma rays, ultrasound and ethyl methyl sulfonate. The manifestation of the mutations started in M_1 but they were mostly expressed in M_2 and M_3 and less in the next generations. The quantity of the harmful to the plants and economically insignificant for mankind mutations was rather higher than that of the useful ones. The useful mutations observed were: shorter stem, earlier maturity, higher leaf surface, larger head and seed size, higher self-fertility, higher 1000 kernel weight, higher seed oil content, variable fatty-acid composition, new CMS sources. The number of the most interesting mutant forms, obtained for the period 1983 - 1994 was 134. Twenty eight new lines were directly produced from mutant forms, originating from varieties Peredovik, Progress, Voronegskii - 272, Trudovik, Start (Russia), Hemus, Vihren and Stadion. Eleven new inbreds, totally differing from their origin were obtained from lines 1607, 2607, 2969, 3004, MF-21 and HA 89. Seven lines were obtained from the hybrids Start (Bulgaria) and NS 26 and 8 lines were produced from 3 synthetics. Three new CMS sources were maintained: 2, coming from the varieties Stadion and Hemus after treatment with gamma rays and 1, coming from Peredovik after treatment with ultrasound.

Key words: Sunflower, mutagenesis, gamma rays, ultrasound, EMS, mutant.

Introduction

Mutagenesis, the artificial inducement of mutatios, has been an important research approach since 1928, when Stadler, Muller and Altenberg showed that X rays and radium cause heritable changes in barley, maize and Drosophila (Bird and Neuffer, 1986). At this moment the problems of the experimental mutagenesis are studied in many crops and many countries in the world. A number of investigations showed that different rates of depression or stimulation of the sunflower growth and increase in seed production could be obtained after treatment with physical and chemical mutagenic factors (Savin and Stepanenko, 1968; Zezjulinski et al., 1969; Kovatchik, 1973; etc.). Under the influence of the mutagenic factors a large spectrum of heritable changes could be induced (Beletskii and Ljashchenko, 1968; Zezjulinski et al., 1969; Tsvetkova, 1970; Plotnikov, 1971; Voskoboinik and Soldatov, 1974; Christov, 1990;

etc.). Different mutant forms with changed morphologic features were produced (Beletskii, 1965; Plotnikov, 1971; Saadat et al., 1974; Sarafi et al., 1974; Luczkiewicz, 1975; Christov, 1990; Christov, 1995; etc.). Important results were obtained concerning the development of forms with changed fatty-acid composition of the oil (Soldatov, 1976; Kubber, 1984, etc.).

A great part of the above-mentioned results support the statement of Anashchenko (1977) that the most important effects of physical and chemical mutagenes are the productions of a large number of recessive genes and cytoplasmic mutations, both of which increase the variability in the cultivated sunflower.

Our study on the utilization of induced mutation is in conformity with the possibilities, means and achievements in this respect and is mainly directed towards the obtaining of practical results - new forms of sunflower plants.

Materials and Methods

The investigation has been carried out for a period of 12 years (1983 - 1994) on the territory of IWS "Dobroudja", the North-East part of Bulgaria.

1. Used biological material - Dry dormant seeds of 13 sunflower cultivars - Peredovik, Progress, Voronegskii-272, Trudovik, Skorospelii, Nadegdnii, VNIIMK-8931, Start (Russia), Balkan, Stadion, Hemus, Vihren and Kutuger; 9 lines - N 49, 24Mit, 130 Mit, 1607, 2607, 2942, 2969, 3004 and HA-89; 2 local forms - N BP-21 and P-13 and 2 hybrids - NSH-26 and Start (Bulgaria) were used in our experiments.

2. Mutagenes and treatment procedures

a) physical mutagenes

- gamma rays - 20 Gy, 40 Gy, 60 Gy, 70 Gy, 80 Gy, 100 Gy, 120 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy, .400 Gy, 420 Gy, 440 Gy and 500 Gy. Co^{60} was used. Dry seeds were treated.

- ultrasound - 0.5 W/cm^2; 1.9 W/cm^2; 2W/cm^2; 2.2 W/cm^2; 2.5 W/cm^2 and 3 W/cm^2 for 2 and 4 minutes. During treatment the seeds were flooded in water, the whole surface well moistened.

b) chemical mutagenes

- ethyl methyl sulfonate (EMS) - 0.2 % and 0.8% for 2 hours. After treatment the seeds were well washed in distilled water for 10 min. A portion of the seeds were dried and aired with hot air for two hours and after that were left to dry further at room temperature.

In all cases and ways of treatment the seeds were planted in the field a day after the treatment. In some occasions part of the treated with gamma rays and ultrasound seeds were planted an year after the treatment – 360 days. Some of the seeds were repeatedly treated with the same dose 360 days later, just before planting on the field. Untreated seeds were used as checks. The trails were carried out in randomized block design in two replicates. The necessary phenologic observations and biometric measurements were done during the vegetation period. Part of the plants, obtained from treated seeds were isolated in preservative bags and were self-pollinated for the production of M_2 progeny. The M_2 seeds from every head were planted as a separate progeny. The selection in M_2 was repeated - before anthesis and after harvesting period. The 1000 seed weight and the seed oil content were evaluated. The selected M_3 seeds from every head were planted as separate progenies. This was the way of proceeding with the following plant generations and seed generations too. When new forms appeared in M_2, M_3, etc., and the stability of the characters was verified in the following 3-4 generations, the mutant forms were registered in the collection of IWS "Dobroudja". The fatty-acid composition of the oil was studied for part of the material from the later generations. The sterile plants, obtained from treated seeds were pollinated with pollen from different varieties and inbreds in order to maintain the sterility. Mutant forms with already established and confirmed characteistics were included as parents in the heterosis breeding for the development of hybrid combinations.

Results and Discussion

1. Utilization of gamma rays for mutation induction.

It was established from the treatment with gamma rays that compared to the other varieties, inbred lines, native forms and hybrids, included in the investigation, highest was the percentage of germinated plants from Peredovik variety. The lower influence of the gamma rays over the seed material from Peredovik cultivar perhaps was due to the stabilization of the variety genotype (Peredovik variety has been used in Bulgaria for seed sunflower production for more than 30 years). Lower percentage of germinated plants was noted at higher doses for the cultivars Progress, Hemus, Vihren, Start, Voronegskii-272, Stadion, Balkan and Trudovik and inbreds N 49, 3004, 1607, 2607 and HA-89 (13% - 32%). This percentage was increased, compared to the checks. The flowering period for Vihren at doses 120 Gy and 150 Gy and for Voronegskii-272, Skorospelii and Start at dose 150 Gy was shorter and started 3 to 5 days earlier than the check. In other occasions - mainly for Hemus variety and inbred 3004 the flowering period had 2 days delay. For many varieties mosaic variegated leaves appeared at 60 - 70 Gy.

With the increase of the treating dose larger patterns appeared more often on the stem, the leaves and heads of different number of plants from Peredovik, Hemus, Progress, Vihren, Start, Voronegskii-272, Stadion, Skorospelii and VNIIMK-8931 varieties and inbreds N 49, 3004, 1607, 2969 and HA-89. The number of treated plants produced

was very low when the doses were highest - from 400 Gy to 500 Gy; in some cases there were one or two plants and no plants in others (Table 1). Many of these plants died at cotyledon stage or during first true leaf stage. The leaves looked like crumpled up into the hollow handkerchief. Many looked like burned (the intensity of these phenomenon depended on the meteorological conditions). Most of the single plants reached inflorescence formation, which had the shape of a fist or bird head without any or with abnormal florets. Few of the M_1 plants developed "normally" from seeds, treated with doses of 350 Gy, 400 Gy, 420 Gy, 440 Gy or 500 Gy. They were mainly from Peredovik cultivar. Few seeds or none seeds were produced from great part of the M_1 plants after self-pollination. This was the problem with the mutant plants, originating from the investigated material, especially at doses above 100 - 150 Gy.

The results obtained after treatment with gamma ray showed that the different genotypes reacted in a different way. The increased doses had a strong lethal influence. Some of the germinated plants in M_2 had yellow-white cotyledons. The coloring of the first true leaves was yellow-green. They soon became brown, withered and the plants died. Such chlorophyll mutations appeared in M_3, M_4 and M_5 too and in some cases even in M_7 generation. Characteristic for those phenomenon was the fact, that chlorophyll mutations could be observed most often when the plants originated from treated seeds with 70 Gy, 100 Gy, 120 Gy and rarer with 150 Gy and 200 Gy. We suppose that great part of them died just during germination.

It was established that some plants, even in M_2 and especially in M_3, M_4, differ greatly from the original material in size, leaf and inflorescence disposition, size and coloring, seed shape and color, branches appearance and seed oil content (Table 2).

Mutations, obtained after treatment with gamma rays, determined as harmful from an agricultural point of view were: the appearance of branched stem - from Start and Peredovik cultivar and native forms BP - 21; degenerated leaves and heads - from Peredovik, Progress, Voronegskii-272, Trudovik, Stadion, Hemus, Balkan varieties, inbred 3004, native forms BP-21 and P-13; inflorescence, lacking disk florets - from Hemus, Progress, Peredovik, Trudovik, Balkan, VNIIMK-8931, Stadion varieties and native form BP-13, etc.

Interesting mutant forms with changed phenotype were obtained from Peredovik, VNIIMK-8931, Start varieties, inbreds N 1607, 2607, 3004 and native form P-13; with changed oil content - from Peredovik, Start, Stadion cultivars, inbreds N 49, 1607, 2607 and 3004; native BP-21 and hybrids NSH-26 and Start (Bulgaria). Mutants with changed fatty-acid composition were produced from Peredovik, VNIIMK-8931, Progress and Start (Russia) varieties; inbreds N 3004, 2607, 1607 and HA-89; and native P-13. Mutants with short vegetation period originated from Peredovik, Progess, Vihren, Start, Trudovik, Voronegskii-272, VNIIMK-8931, Stadion varieties; and inbreds N 49, 1607 and 3004 (Table 2 and 3).

A new form was obtained from VNIIMK-8931 at a doses of 150 Gy in M_5 - a line,

which differs from VNIIMK-8931 in the size of the whole plant; the nervation and color of the leaves; with a knee on every petiole, 2 cm from the blade. At an early stage of development the sunflower plant resembled an ornamental cabbage form. Other interesting morphologic changes were registered in mutants, originating from Start variety - some sections appeared on the stem, looking like internodes.

White seeds of P-13 with grayish-black stripes were treated with 120 Gy and 150 Gy and as a result some forms were obtained with grayish-black and black seeds. Plants were obtained from inbreds N 49, 1607, 2607 and 3004, 30-50% higher than the initial material. Besides, forms were registered with considerably shorter stem than the initial inbreds 1607, 2607, 3004 and 2942. Some of the mutants from inbreds N 49, 130, 1607, 2607, 3004 and HA-89 differ in their larger seeds, higher oil content and higher 1000 seed weight. Other mutants were obtained from the same inbreds, but with smaller seeds and 1000 seed weight and with a very good self-fertility.

New forms with higher stem, larger in size and greater in number leaves, larger head and longer vegetation period were produced in M_3 from inbred 3004, treated with 150 Gy. Some of them differ in the peel color and the seed shape and size. The seeds were longer, thin, "spindle-shaped" and gray with black stripes.

Forms with male sterile inflorescence were other interesting mutations. Twenty-nine plants with male sterile inflorescence were produced until 1995. The male sterility was maintained just in four of them. The first maintained MS was obtained in M_1 of Stadion variety (dose of treatment - 70 Gy) in 1984; the second - in M_1 of Hemus variety (dose of treatment - 150 Gy) in 1986. The third and fourth MS were obtained in M_6 and M_5 of Peredovik variety in 1991 and 1992 respectively. For the first two ones there was approved they were of cytoplasmic type. The last two male sterilities are still in study.

In 1991 six male sterile plants were produced from inbred N 2607 and 2 - from inbred N 3004. Two MS inflorescence from the mutant material of inbred 2607 were pollinated with pollen of inbred N 2607 (no treatment). Only one of the mutant MS inflorescence of inbred 3004 was pollinated with pollen of inbred 3004 (untreated). In both cross combinations interesting results were obtained in F_2 and especially in F_3. It became evident, that the gamma rays influenced great number of characters - plant height, vegetation period, seed size, peel color, seed oil content, etc. All those results showed that by treating sunflower seed material with gamma rays, undesired as well as desired, though few in number mutations could be induced, which give the opportunity to enrich the sunflower variability.

2. Utilization of ultrasound for mutation induction.

Seeds of Peredovik variety and inbreds N 24 Mit, 130 Mit, 2969, 3004 and HA-89 were treated with the purpose to investigate the possibility to induce mutations by ultrasound. It was established that the percentage of germinated plants from treated with

ultrasound seeds was lower than that of the check. The differences were very clear when the seeds were twice treated with 2.2 W/cm^2, 2.5 W/cm^2 and 3 W/cm^2 and duration of 4 minutes. The ultrasound influence on M_1 plants was almost invisible. Differences between the plants, originating from the inbreds and cultivar Peredovik could be observed in M_2 and M_3 at the earliest.

The morphological differences became clearer in M_4. Under the ultrasound influence mutant plants were produced, which differed in the size of the stem, leaves, inflorescence and seeds, in the leaf and seed shape and number, and the vegetation period. This was especially true for the mutant material, coming from inbreds 130 Mit, 2969, 3004 and cultivar Peredovik (Table 4). Some mutants were obtained from inbred 3004, similar to those from gamma ray treatment - they had high stem, large leaves, long vegetation period and long, thin seeds. There were changes also in the seed oil content and at lower extent - in the fatty-acid composition of the oil (Tables 4, 5).

The most interesting mutation, obtained under the influence of ultrasound was the CMS from cultivar Peredovik. A double treatment was conducted. The first one was conducted with an intensity of ultrasound - 2 W/cm^2 and 2 minutes (April, 1984). After the treatment the seeds were dried at room temperature and stored in a dark and dry place. The second treatment was carried out in April, 1985. The ultrasound had an intensity of 2 W/cm^2 and exposure - 4 minutes. The seeds were sowed in field conditions one day after the second treatment. In 1987 three plants developed normally in M_3 generation in one of the entries (N 141). Two of the inflorescence were pollinated separately with a normal pollen from lines 3853 and 2934, and 429 and 211 seeds were produced respectively. 100% sterile plants were produced from these seeds. The results showed that mutant material could be produced under the influence of ultrasound, but better were the results when higher doses or double treatments were used.

3. Utilization of Ethyl Methyl Sulfonate (EMS) for mutation induction.

Ethyl Methyl Sulfonate (EMS) was used in order to induce mutations, using chemical mutagenes. Seeds of inbreds N 1607, 3004 and HA-89 were treated. It was established that the number of germinated plants was 10-18% lower, compared to the check, when EMS in 0.8% concentration was used. Moreover, a delay in the germination of the plants was observed. Several branched plants were obtained in M_1 from the seeds of the three investigated inbreds. Degenerated plants were obtained from inbreds N 1607 and HA-89 - with 3-4 accreted inflorescence. Differences in the seed oil content were observed in M_2.

Morphological differences were noted in M_2 and M_3. Changes in stem height, in size of stem, leaves and inflorescence, in vegetation period, in seed oil content were registered. In some cases there were differences even in fatty-acid composition of the oil. Some of the results are presented in tables 6 and 7.

Conclusions

The summarized results, obtained by using the experimental mutagenesis showed that sunflower mutations could be induced by treating seed with gamma ray, ultrasound and EMS. Along with the undesired such mutations were obtained, which had economical significance in sunflower utilization. Besides short stem and early matured forms there were obtained some with higher seed oil content or changed fatty-acid composition of the oil. The number of interesting mutant forms, produced until 1994 was 134. Twenty-eight inbred lines were developed directly from mutant forms, with Peredovik, Progress, Voronegskii-272, Trudovik, Start (Russia), Hemus, Vihren and Stadion origin. Totally new eleven lines, produced from inbreds N 1607, 2607, 2969, 3004, native-21 and HA-89 differed greatly from their origins.

There were obtained 7 new lines (R-lines) from Start (Bulgaria) and NSH-26 hybrids and 8 lines from 3 synthetics. Three new CMS-sources were maintained - two from Stadion and Hemus varieties after gamma ray treatment and one from Peredovik variety, after ultrasound treatment. The best mutant lines and these three CMS-sources were used in heterosis selection for developing the new hybrid combinations.

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Table 1. Results on the influence of gamma rays on Peredovik seeds and the plants obtained from them in 1991 (a) and 1992 (b).

Doses Gy	Year a,b	Planted seeds N	Germinated seeds N	After 10 days N	After 20 days N	After 35 days N	Plants, reaching anthesis N	Plants with seed set N
check	a	84	78	78	78	78	78	78
	b	126	122	122	122	122	121	121
60	a	84	71	71	71	70	70	70
80	a	84	72	68	68	68	66	66
	b	126	97	97	96	96	90	78
100	a	84	62	62	62	62	62	60
	b	126	92	90	90	90	84	82
150	a	84	67	66	66	66	66	66
	b	126	101	98	98	98	97	97
200	a	84	45	45	45	45	44	41
	b	126	103	103	101	89	89	82
250	b	126	94	94	91	91	90	89
300	a	84	32	18	16	15	15	11
	b	126	90	84	76	76	68	52
350	b	126	48	40	18	10	10	10
400	a	84	10	1	1	1	1	0
	b	126	32	21	8	8	8	5
420	b	126	59	18	9	8	3	3
440	a	84	8	0	0	0	0	0
	b	126	33	12	7	4	4	3
500	b	126	27	19	5	2	2	2

Table 2. Characterization of some mutant sunflower forms, obtained by treatment with gamma rays, 1994.

Material N	Plant height (cm)	Head diameter (cm)	1000 seed weight (g)	Oil content (%)	Vegetation period (days)
Peredovik	**205**	**26**	**71**	**45.35**	**122**
161	147	21	65	41.32	108
780	160	22	45	45.76	111
784	155	23	50	43.23	109
Start	**170**	**18**	**78**	**42.15**	**116**
226	144	20	75	45.33	115
228	150	21	75	46.90	112
230	155	22	78	46.00	113
VNIIMK-8931	**225**	**26**	**73**	**41.98**	**120**
671	155	28	58	43.85	118
672	140	26	48	44.57	106
673	145	26	48	43.39	106
675	155	26	65	45.60	112
Vlhren	**165**	**29**	**70**	**41.50**	**118**
220	105	26	86	41.05	106
221	130	25	80	43.11	105
MF P-13	**235**	**35**	**110**	**29.50**	**131**
337	160	28	95	46.88	125
339	145	25	74	48.37	123
357	140	27	90	48.19	123
L-1607	**110**	**20**	**68**	**38.37**	**110**
617	105	21	88	44.16	109
852	105	22	68	41.97	105
854	100	21	62	42.89	104
L-3004	**120**	**19**	**76**	**39.18**	**114**
265	190	20	48	44.42	131
271	145	21	64	48.11	120
272	165	20	72	47.32	119
283	100	23	76	40.12	111

Table 3. Results on the study of fatty-acid composition in the seed oil of mutants, obtained by treatment with gamma rays, 1994.

Material N	Oil content (%)	Linoleic acid (%)	Oleic acid (%)	Stearic acid (%)	Palmitic acid (%)
Peredovik	**45.35**	**61.7**	**27.1**	**4.7**	**6.5**
156	46.16	**70.6**	19.6	4.2	5.6
159	46.07	52.1	35.8	5.1	7.0
642	37.34	**70.3**	18.9	1.8	**9.0**
643	**50.01**	38.6	47.7	4.6	**9.1**
Start	**42.15**	**54.7**	**28.8**	**8.5**	**8.0**
225	**46.90**	35.4	**46.5**	**11.6**	6.5
226	**45.33**	37.9	**42.8**	**12.4**	6.9
229	**45.72**	34.8	**46.5**	**11.9**	6.8
590	**50.08**	**70.0**	21.0	3.0	6.0
596	**46.29**	39.8	**43.8**	**10.9**	5.5
L-1607	**38.37**	**61.5**	**27.9**	**4.7**	**5.9**
684	**43.10**	54.9	**33.2**	8.2	3.7
852	41.97	42.6	**40.4**	**8.5**	8.5
853	41.38	51.0	**36.1**	5.4	7.5
L-2607	**41.43**	**59.9**	**28.2**	**4.8**	**7.1**
712	43.00	54.7	31.1	7.5	6.7
716	41.36	**70.2**	20.5	3.1	6.2
719	**44.88**	**71.2**	17.4	3.4	8.0
859	**45.25**	54.5	**33.7**	5.8	6.0
1078	**45.01**	46.7	**36.1**	7.9	**9.3**
L-3004	**39.18**	**65.9**	**20.6**	**6.9**	**6.6**
273	40.74	**73.1**	21.4	3.5	5.3
277	36.65	**74.0**	15.9	3.0	7.1
279	39.01	**76.4**	15.2	4.0	4.4
1089	**43.21**	**71.3**	16.1	5.6	7.0
1095	41.79	34.4	**54.9**	5.5	5.2

Table 4. Characterization of some mutant sunflower forms, obtained by treatment with ultrasound, 1995.

Material N	Plant height (cm)	Head diameter (cm)	Leaf size Length/Width (cm)	Seed oil content (%)	Vegetation period (days)
Peredovik	**205**	**26**	**24/23**	**46.45**	**122**
951	155	27	29/24	44.76	113
954	165	28	27/25	42.90	115
958	210	22	29/27	44.81	118
HA-89	**110**	**22**	**27/26**	**49.82**	**116**
961	80	25	29/27	49.76	108
966	90	25	28/24	49.31	108
L-2969	**125**	**20**	**18/13**	**38.92**	**102**
972	155	22	28/21	42.30	106
973	160	26	26/22	44.04	104
974	152	22	23/17	43.29	103
L-3004	**139**	**21**	**26/19**	**38.48**	**114**
977	210	26	29/23	42.62	122
978	180	22	26/21	39.23	119
979	190	23	28/25	36.97	128

Table 5. Results on the study of fatty-acid composition in the seed oil of mutants, obtained by treatment with ultrasound, 1994.

Material N	Oil content (%)	Linoleic acid (%)	Oleic acid (%)	Stearic acid (%)	Palmitic acid (%)
Peredovik	**45.35**	**61.7**	**27.1**	**4.7**	**6.5**
732	46.00	58.7	28.5	5.3	7.5
733	**47.27**	58.2	28.2	5.6	8.0
734	44.76	**67.1**	24.6	5.2	3.1
735	44.60	57.3	27.6	7.2	7.9
HA-89	**48.32**	**57.8**	**29.0**	**5.8**	**7.4**
738	**50.09**	47.4	**39.4**	5.7	7.5
739	48.18	61.9	29.6	5.0	3.5
740	**50.21**	59.8	28.6	5.1	6.5
L-2969	**38.41**	**69.0**	**22.7**	**2.8**	**5.5**
742	40.60	58.0	**30.9**	4.5	6.6
743	42.93	62.0	24.0	6.3	7.7
744	**44.04**	51.7	**35.9**	8.5	3.9
L-3004	**39.18**	**65.9**	**20.6**	**6.9**	**6.6**
745	**42.25**	59.8	**26.5**	7.1	6.6
746	**42.67**	61.7	24.0	8.1	6.2

Table 6. Characterization of some mutant sunflower forms, obtained by treatment with EMS, 1995.

Material N	Plant height (cm)	Head diameter (cm)	Leaf size Length/Width (cm)	Seed oil content (%)	Vegetation period (days)
HA-89	**110**	**22**	**27/26**	**49.82**	**116**
818	205	28	33/30	44.84	125
819	210	24	29/25	42.05	122
823	120	22	28/26	47.91	116
829	125	19	26/24	**50.27**	115
830	120	29	30/25	48.69	115
841	150	25	24/27	**50.68**	116
844	145	25	26/27	45.32	111
L-1607	**135**	**22**	**32/27**	**40.39**	**110**
852	110	22	28/26	41.30	109
856	115	25	32/30	41.33	111
858	115	20	30/25	44.28	111
859	116	22	28/22	45.14	106
L-3004	**139**	**21**	**26/19**	**38.48**	**114**
2124	150	25	25/24	44.38	116
2127	165	24	27/23	45.75	113
2128	170	26	28/23	45.22	113

Table 7. Results on the study of fatty-acid composition in the seed oil of mutants, obtained by treatment with EMS, 1994.

Material N	Oil content (%)	Linoleic acid (%)	Oleic acid (%)	Stearic acid (%)	Palmitic acid (%)
HA-89	**48.32**	**57.8**	**29.0**	**5.8**	**7.4**
666	**51.36**	50.1	**37.6**	6.0	6.3
672	**50.27**	57.7	31.2	4.8	6.3
676	48.97	44.8	**38.4**	7.2	**9.6**
680	49.82	63.2	22.2	6.4	8.2
682	49.02	48.3	**35.5**	8.6	7.6
1081	**50.88**	**72.1**	18.3	3.5	6.1
1083	**52.81**	**70.4**	18.5	4.1	7.0
L-1607	**38.37**	**61.5**	**27.9**	**4.7**	**5.9**
684	**43.10**	54.9	**33.2**	8.2	3.7
685	41.33	59.4	31.7	4.0	4.9
686	**45.14**	50.9	29.7	**11.2**	8.2
688	37.37	56.6	27.1	6.4	**9.9**
L-3004	**39.18**	**65.9**	**20.6**	**6.9**	**6.6**
1084	**49.01**	60.3	25.0	5.4	**9.3**
1085	**50.55**	62.0	**27.2**	4.6	6.2
1086	**47.59**	60.0	**29.1**	5.2	5.7