Mutagenesis Breeding of Seabuckthorn (*Hippophae rhamnoides* L.)

G.F. Privalov, L.P. Solonenko and G.M. Skuridin

Institute of Cytology and Genetics of Siberian Department of the Russian Academy of Sciences Novosibirsk 630090, Russia (E-mail: skuridin@bionet.nsc.ru)

ABSTRACT

Seabuckthorn (Hippophae rhamnoides L.) plants are highly resistant to the ionizing irradiation. Seedling viability decreased considerably at a dose higher than 50 kR. Reduction in 50% viability was observed at the dose of 60 kR. Irradiation at the dose of 30 kR, significantly raised the frequency of visible chromosome aberrations. A part of aberrations was invisible, because of small chromosome size. So we suppose that the dose of 60 kR is sufficient to obtain 50% aberrant cells. Most cells became aberrant at the dose of 110 kR. It is of interest that gamma- irradiation led to earlier flowering (at the age of 4 years) and stimulated flowering in male plants, by 2-3 times than in the female plants. Plant growth stimulated at the doses of 1-5 kR, whereas seed germination was stimulated at the doses of 10-20 kR. The experimental data obtained indicated that the sensitivity of seabuckthorn to chemical mutagens is genotype-dependent. Various morphological abnormalities were induced, such as shoot bifurcation, double and small-crinkled-leaves. The total abnormality frequency increased by 5-8 folds under the effect of these treatments, and the part of M, plants carrying valuable quantitative abnormalities, increased about 4 times over the controls. On the contrary, the low mutagen concentrations produced stimulating effects. On higher germination and general resistance to hazardous factors, enhanced plant growth and development. The other results of treatment with high doses included the lower fruit weight and higher mean content of sugars. The mutations, induced in seabuckthorn, like in other plant species, affect the shape and colour of leaves, fruit and shoots, the growth rate in height, internode length, chlorophyll content in leaves, adaptation of plants, their response to temperature, day-length and other characters. Phytohormonal treatment of seeds with gibberellic acid led to arising of male F, progeny plants, bringing fruits two times more than the controls. It is interesting that the exceptional parthenocarpic form, carrying rudimentary seeds, was obtained for the first time by phytohormonal treatment of this genotype. A part of the treated plant, demonstrated fruit setting without seeds.Subsequent treatment of this form with gibberellic acid led to a fruit size and setting increased close to the initial 'Ziryanka' variety, but seedless. After the exposure of seeds to EMS, carotenoid content in the fruit of the M] plants decreased with increasing mutagen concentration, from 14.5 mg% in the control to 10.5 mg% at an EMS concentration of 1.0%. Under the effect of ionizing irradiation, individual and group variability of plants, for fruit oil content widens. In the control, individual variability ranged from 2.8 to 8.4%, after the treatment of seeds with gamma-rays, variability widened from 1.1 to 9.3%. Treatment of seabuckthorn seeds at high doses (from 30-100 kR) also widened the individual viability range for fruit sugar content. Treatment of "Ziryanka" seeds with gamma-irradiation at a dose of 10-25 kR certainly decreased the mean value of ascorbic acid from 129 mg% in the control to 92 mg% in experimental variants. Under the effect of treatment of seeds with gamma rays, the range of individual variability in the weight of 100 fruits increased from 20.0-46.6 g in the control to 14.5- 48.7 g in the experimental series. The range became still wider under the effect of seed treatment with EMS.

Key words: Seabuckthorn, mutagenesis, "Ziryanka" variety, gamma irradiation growth, oil, sugar and vitamin C.

INTRODUCTION

According to the FA O/IAEA data, more than 1,200 mutant cultivars have been obtained from direct use of induced mutants upto 1989. About a half of these cultivars have been obtained by direct propagation and selection of the induced mutants, the rest were the result of efficient cross breeding. The improved characters of the mutants included, yield increased, early ripening, better quality and technological properties of yield, higher tolerance to the unfavorable environmental conditions etc. During the past decades, geneticists and breeders have applied much efforts to the develop efficient methods for controlling the mutational plant variability, while using various mutagenic agents. IAEA in 1970 and the second edition in 1977 published the first compilation "Manual on Mutation Breeding". They give a good idea of the first released varieties and methods, and of all the early useful contributions to the experimental mutagenesis and mutagenic research.

The method of experimental mutagenesis and mutation breeding has been widely applied in self-pollinating plants. Seabuckthorn is a cross-pollinated, dioecious woody plant and there was little success in this species, because it was difficult, even not feasible to apply standard mutagens. However, seabuckthorn has some advantages in the mutation breeding:

a) Both dominant and recessive mutations can be phenotypically evaluated as early as in the M2, due to their natural heterozygosis;

b) Vegetative propagation, which is a tool for the development, maintenance and reproduction of the obtained genotype,

c) Easy mutagen treatment, observations and other procedures due to large plant size.

The studies on the experimental mutagenesis in seabuckthorn have started at the Institute of Cytology and Genetics (Novosibirsk, Russia), since 1959. The research program included:

1) Analysis of the sensitivity of seabuckthorn seeds to the ionizing irradiation and chemical mutagens.

2) Development of methods for the identification of mutations in the M1

3) Analysis of the frequencies and patterns of induced mutations in the M1 .

4) Screening for the possibilities of using induced mutants in the breeding work.

SENSITIVITY OF SEABUCKTHORN SEEDLINGS TO THE MUTAGENS

Sensitivity to gamma-irradiation

Seabuckthorn plants are highly resistant to the ionising irradiation (Privalov, 1968). Seeds collected from the wild plants of a natural seabuckthorn population grown in Altai, after gamma irradiation, retained their germination ability up to a dose of 100 kR gamma-rays. However, seedling viability decreased considerably at a dose higher than 50 kR (Table 1). Reduction in 50% viability was observed at the dose of 60 kR (Privalov and Shmeleva, 1971). The similar results for gamma- treated (0.5-60.0 kR) seeds of “Maslichnaya” variety were obtained by Eliseev and Mishulina (1974).

Table 1. Effect of high doses of gamma-irradiation on seabuckthorn seedlings

Dose (kR)	*Germination in in soil (% of control)*	*Seedling survival (% of germinated*)*	*Plant height (% of control**)*	*No. of Plants flowering (%***)*	*No. of chlorophyll deficient plants (%)*	*No. of adult plants studied*
Control	100	100	100	18.1	0	94
30	72	87	94	43.0	0	93
50	53	65	82	42.4	5	172
70	22	50	76	39.6	8	101
100	10	26	69	48.0	20	50

(*at the age of 4 months; **at the age of 2 years; ***at the age of 4 years).

Cytological investigations of gamma-rays treated seedlings, showed that irradiation at the dose of 30 kR, significantly raised the frequency of visible chromosome aberrations. A part of aberrations was invisible, because of small chromosome size. Therefore, we suppose that the dose of 60 kR is sufficient to obtain 50% aberrant cells. Most of cells became aberrant at the dose of 110 kR (Table 2) (Privalov and Shmeleva, 1971).

Table 2. Effect of high doses of gamma-irradiation on seabuckthorn embryonic root cells

Dose(kR)	*No of embryonic roots studied*	*No of mytotic cells studied*	*No of cells with chromosomal aberrations (%)*
Control	10	396	2.8±1.1
30	11	497	9.6±1.6
60	5	227	40.9±6.8
90	4	171	54.4±1.4
110	9	611	62.0±3.8

Therefore, the final plant survival depends on the degree of intracellular compensation ability and further somatic selection within the seedling tissues. The radiation syndrome of the M, seabuckthorn plants results in a decrease in height, as a general characteristic. However, except small height and rare changes in leaf colour (yellow-green), these juvenile plants were similar to controls. As the data in Table 1 show, earlier flowering onset was induced by the treatment of seeds with a dose of 30-100 kR. The number of flower-forming plants increased by 20-30%, as compared to the control. The fertility of plants somewhat decreased, as compared to the normal, however, most seeds remained viable. It is of interest that gamma-irradiation led to earlier flowering (at the age of 4 years) and stimulated flowering in male plants, by 2-3 times than in the female plants.

It was established that gamma-irradiation at lower doses (less than 20 kR) promoted the growth and development of seedlings (Table 3). Later it was established, that the optimum irradiation doses to obtain maximum effects in seabuckthorn depend on a number of factors, including genetic differences and physiological conditions of seeds. Furthermore, these are dependent on the characteristics chosen, for example, plant growth stimulated at the doses of 1-5 kR, whereas seed germination was stimulated at the doses of 10-20 kR.

Table 3. The effect of low doses of gamma-irradiation on seabuckthorn seedlings

Gamma-irradiation (kR)	*Germination in soil (% of control)*	*Seedlings survival (% of control*)*	*Plant height (% of control**)*	*Number of adult plants studied*
Control	100	100	100	53
1	123	93	110	112
5	107	96	112	78
10	303	103	105	46
15	303	99	98	202
20	179	100	99	77

(*at the age of 4 months; **at the age of 5 years).

Sensitivity to chemical agents

It was established that in seabuckthorn, resistance to chemical mutagens is not related to its resistance to gamma-irradiation. We have found that treatment of seeds derived from some plants significantly increased yield, survival and growth of seedlings in height, whereas treatment of those collected from others, by contrast, decreased these values. The experimental data obtained indicated that the sensitivity of seabuckthorn to chemical mutagens is genotype-dependent (Table 4) The results obtained by Eliseev et al (1982, 1988) were almost the same.

Treatment with chemical mutagens, at high doses resulted in growth inhibition, as amply confirmed by the data in the literature. In application to seabuckthorn, the doses were neither critical nor lethal (Potapov et al., 1978; Eliseev et al., 1982, 1988). We also found that the optimum dose of a chemical mutagen varies widely and that is never lethal.
High doses of chemical mutagens, like nitrosomethylurea (NMU), nitrosoethylurea (NEU), dimethylsulfate (DMS), ethyleneimine (EI) and 1,4-diazoacethylbutane (DAB), may enhance the individual variability in plant height, intemode length and leaf index. Various morphological abnormalities were induced, such as shoot bifurcation, double and small-crinkled-leaves. The total abnormality frequency increased by 5-8 folds under the effect of these treatments, and the part of plants carrying valuable quantitative abnormalities, increased about 4 times over the controls. (Potapov et al., 1978). On the contrary, the low mutagen concentrations produced stimulating effects. Higher germination and general resistance to hazardous factors, enhanced the plant growth and development. Similar stimulation phenomena by low mutagen doses, were observed in various perennial woody species (Belikova, 1982). Our data on various mutagen treatments of seeds with EMS (0.5% aqueous solution, 24 hrs), demonstrated obvious stimulation of one-year seedlings (Privalov et al., 1983).

Table 4. Variations in seabuckthorn seedling characteristics

Treatment	*Laboratory germination (%)*	*Field germination (%)*	*Survival of one-year-old seedlings (%)*	*Height of one-year-old plants (cm)*
Control	74.0-100	18.0-43.0	12.9-25.9	11.6-15.8
Nitrosomethylurea (0.012%)	63.2-95.5	26.0-48.1	12.4-43.3	10.1-19.1
Ethylenimine (0.02%)	82.0-95.5	16.0-58.3	9.9-53.0	11.9-20.7
Dimethylsuphate (0.05%)	79.5-100	17.5-58.6	13.1-44.1	12.3-21.4

Note: Seeds from nine maternal plants were soaked in a solution of chemical mutagens at appropriate concentration for 18 hours.

LONG-TERM EFFECTS OF MUTAGEN TREATMENT IN THE M1 PLANTS

Growth of M, seabuckthorn plants was mainly suppressed during the first growth. Some plants eventually died. As a rule, survived plants overtake controls some years later. The stimulation effects of low doses of irradiation and chemicals on plant growth and development may retain or develop 4-5 years later and they are manifested in characteristics of adult plants. For example, fruit pulp is a maternal plant tissue, therefore its chemical composition may “invisibly” contribute to the consequences of mutagen treatment of seeds. In fact, certain chemical characteristics of fruit pulp show definite changes. It was established that low doses of ionizing irradiation did not lead to appreciable changes in fruit pulp composition of M| adult plants (Table 5). They produced a trend to lower the ascorbic acid and total carotenoids content (Skuridin et al., 1998).

Table 5. Effect of low doses of gamma-irradiation on the fruit pulp main characteristics in the M1 plants

Dose (kR)	*No of plants studied*	*100 fruit weight (g)*	*Sugar content (%)*	*Oil content (%)*
Control	14	27.4	8.3	6.0
1-5	45	27.5	8.0	5.9
10.20	49	26.0	7.8	6.0

The other results of treatment with high doses included the lower fruit weight and higher mean content of sugars (Table 6). One reason for the differential viability of juvenile seedlings is possibly due to the, as yet, unrealised characteristics of adult plants (Privalov and Solonenko, 1977). Another possible reasons are the long-term modification effects, retained through successive vegetative cycles (Skuridin et al1998), which may be elucidated through further advance research.

Table 6. Effect of high doses of gamma-irradiation on fruit pulp main characteristics in the M1 plants

Dose (kR)	*Weight of 100 fruits (g)*	*Sugar content (g)*	*Dry substances (%)*	*Oil content (%)*
Control	33.6	2.2	14.8	4.2
30	26.5	2.4	14.5	4.2
50	27.2	2.8	15.0	4.0
70	29.0	2.7	14.8	4.1
100	27.8	2.8	15.1	4.1

IDENTIFICATION OF SOMATIC MUTATIONS IN THE M1 PLANTS

When genetically identical material is treated with a mutagen, uncertain variability, conforming to Vavilov‘s law of homologous series in variation arises (Vavilov, 1922). If the material is genetically not identical, individual variability expresses at any given time, show both initial and induced variability. When seeds are treated, it is impossible to know for certain, which and how many genes have been affected. Moreover, it is impossible to treat just one embryonic cell, to grow into a whole mutant organism. A mature seed contains thousands of cells exposed to mutagen at the same time. The resulting plant is not the whole genotype, rather an aggregate of genetically different cell clones^ This leads to endogenous genetic variability in the adult plant affecting morphological, biochemical, physiological and other characters. Such plants are called chimeras. They remain chimeric through successive vegetative cycles. This must be remembered for subsequent vegetative propagation of the mutant seabuckthorn plants. Under the effect of mutagen on the embryonic tissues, two types of changes arise in plants grown:

1. Transient changes, affecting the normal morphogenesis and leading to the appearance of morphosis, for example.

2. Long-term changes, resulting in the formation of genetically heterogeneous tissues and organs, i.e. chimeras.

The changes are transient, because plants consist of the meristematic tissues of two types, tissues with limited growth, from which leaves, flowers and fruit are derived, and tissues with unlimited growth at the apical vegetative cones of shoots. When mutations arise in cells from which tissues with limited growth derive, mutational changes in the phenotype are manifested only once, i.e. the year after seeds or buds were treated with a mutagen. During the second and subsequent years of vegetation, mutation manifestation depends on the presence of mutant cells in the apical vegetative cones of the shoots, i.e. in tissues with unlimited growth. The cloning of leaves and other organs in the sterile culture may identify the mutations induced in tissues with limited growth. The mutations induced in the tissues of chimeric plants, may be recovered by strategies counteracting the formation of chimeras by “dechimerization”. The strategies are as follows:

1. Repeated cloning of shoots (or roots) by cutting branches (grafting) or by other ways and means of vegetative propagation;

2. Identification of mutations, by stimulating the development of root shoots from resting ("sleeping") buds, placed on the basal zone of the stem.

The experiments, we performed with seabuckthorn and other woody plant species, demonstrated that the successful identification of somatic mutations in the M, chimeric plants is dependent on the knowledge of the genetics of the characters, of their phenotypic expression and also on the experience of the experimenter. It was observed that most intense somatic selection resulting in elimination of mutant cells is most efficient, when applied early in the plants grown from mutagen-treated seeds. In this case, up to 80% of all the mutant clones remain on the basal zone. These mutations may be retained for many years until conditions become favourable for individual shoots and later trees to develop from them. More intense cloning of the chimeric plants raised the probability, that somatic mutations will be "unmothered", i.e. release from the maternal influence.
The identification of mutations, in the resting buds of the root meristem of the tree stem, is advantageous, because, in this way, mutant plants can form from the same root system next to the primary genotype. This provides optimum conditions for studying the differences between the mutant and primary the forms during their development. The ontogeny of the M mutants consists of vegetative generations (the V,, V2, V3 and subsequent generations) of an isolated clone of a single mutant following its own pathway. The task to inherit mutations is obligatorily fulfilled by the sexual process, in the case of generative mutagenesis, by contrast, it is fulfilled through successive vegetative generations (M,V,, M,V,, M,V3 and the next generations), not obligatorily by the sexual process, in the case of somatic mutagenesis. As our previous studies have shown, the percentage of true somatic mutations is about 50% of all the M, induced mutants, which, however, retain their characters, when propagated vegetatively (Privalov, 1968).

MUTATIONS OF PARTICULAR CHARACTERS OF SEABUCKTHORN

The pattern of the observed induced mutations, is identical to that of spontaneous mutations, observed in the natural populations. The method of induced mutagenesis has an advantage, not to be overlooked. Even when the primary material is limited, the mutagen-induced sharp increase in the mutability, allows to obtain mutations, rarely occurring in nature and withstanding the strong pressure of natural selection, as well as to induce novel mutations, meeting the requirements of their cultivated counterparts. Vavilov's law of homologous series in variation holds true for all the plant species. Indeed, as Vavilov observed, genetically close species and genera show similar homologous series in inherited variation with such a strict regularity that, knowing certain forms within a species, parallel forms, within other species or genera can be predicted. Induced mutations in various species and genera of fruit and woody plants obey Vavilov's law of homologous series in variation (Privalov, 1966). The mutations, induced in seabuckthorn, like in other plant species, affect the shape and colour of leaves, fruit and shoots, the growth rate in height, internode length, chlorophyll content in leaves, adaptation of plants, their response to temperature, day-length and other characters. The most frequently visible types of induced mutations in seabuckthorn are described in Table 7.

Chlorophyll mutations of various types, arose under the effect of the studied mutagens, ranked first in frequency (10-15%). Induced mutations, that affected the growth rate and length of internodes ranked next, had frequencies varying from 3-8%. Dwarf and semi-dwarf mutations arose with a frequency of 1%. The frequencies of the other mutations were less than 1% of the number of survived plants. Fruit yield of most mutants was decreased, as compared to the normal forms. However, fruit yield could be completely recovered by changing the genotypic environment. It could be even increased, under the effect of monohybrid heterosis and gene position. To correctly evaluate the importance of the mutations in the breeding, it is desirable to study them not only in the homozygous, but also in the heterozygous condition.

Table 7. The major types of seabuckthorn somatic mutations in the M1 induced though ionising irradiation and chemical mutagens

Symbol	*Designation*	*Description*
A	Albino	Cotyledons and leaves white, without chlorophyll
L	Light green	Cotyledons and leaves pale green, became dark green by the end of vegetation and with age
Nld	Narrow-leafed-dwarf	Dwarf and semi-dwarf plants with narrower leaves
G1	Glossy leaf	Leaves glossy, multisquamous
Y	Yellow	Cotyledons and leaves pale yellow, pale green by the end of the vegetation
Dg	Dark green	Leaves dark green
Brl	Broad-leafed	Leaves broad

Thrl	Thornless	Thornless or few thorns
Br	Brachytic	All the parts of the plant shortened, leaves dark, crinkled
Lf	Large-fruited	Fruit large
Rf	Red-fruited	Fruit red

The effect of mutagens on the sex characters

Seabuckthorn is a dioecious plant, in which the normal male to female ratio is 1:1 in the case of seed propagation. Cytogenetic studies have demonstrated that in seabuckthorn, sex is determined by the sex chromosomes in the karyotype. Homogametics are females (XX) and heterogametics are males (XY). The male sex chromosome (Y) is smaller than the female (X) (Shchapov, 1979). Nevertheless, hermaphrodite flowers sometimes develop on the normal male plants.

Eliseev had reported about sex mutant plant, induced by exposing seabuckthorn seeds (var. Dar Katuni) to gamma rays at 30 kR. This mutant seems to be a mosaic, in which both male and female fertile flowers formed together and contained viable seeds. Thus, he obtained artificially a monoecious plant by mutagen treatment. This mutant would be useful for the further genetic researchers and breeders (Eliseev, 1985a). We found previously a number of hermaphrodite and sterile seabuckthorn plants and presently in the F2 offspring of mutant variety “Ziryanka”. Phytohormonal treatment of seeds with gibberellic acid led to arising of male F2 progeny plants, bringing fruits two times more than the controls (Lbova,1998).

It is interesting that the exceptional parthenocarpic form, carrying rudimentary seeds, was obtained for the first time by phytohormonal treatment of this genotype. A part of the treated plant, demonstrated fruit setting without seeds. Subsequent treatment of this form with gibberellic acid led to a fruit size and setting increased close to the initial ‘Ziryanka’ variety, but seedless. It is well propagated by the grafting without loss of parthenocarpy (Shchapov, 1999). This is the clearest evidence, that a latent mutation was present in this plant material in a sectorial chimeric state (Shchapov and Kreimer, 1998). No sex mutants were obtained by the chemical mutagenesis of seabuckthorn, so far.

The effect of mutagens on the carotenoid content

The factor, determining the carotenoid content in the fruit, is genetically conditioned colour. There are ample data in the literature indicating that carotenoid content is highest in red fruit and lowest in yellow, the content is minimal in the milky-white fruit of the Caucasian population (Eliseev et al., 1976; Korzinnikov et al., 1983; Muraviova and Lagazidze, 1985). It is generally accepted that the great majority of H.rhamnoides fruits are orange, showing a transition from yellowish- orange to reddish-orange. As the data, we obtained show; the maximum carotenoid content in red-fruit seabuckthorn half-sibs is two fold higher than that in their yellow counterparts, being 29.3-30.3 mg% and 14.0-14.4 mg%, respectively. Orange fruit is intermediate between the red and yellow (19.1-20.0 mg%).

The degree of individual plant variability is extremely wide. It reaches to 2.2-26.9 mg% carotenoid, even within related half-sibs (F1) offspring. After the exposure of seeds to EMS, carotenoid content in the fruit of the M, plants decreased with increasing mutagen concentration, from 14.5 mg% in the control to 10.5 mg% at an EMS concentration of 1.0 % (Table 8). It is interesting that the decrease in carotenoid content, is due to lower number of plants, carrying fruits with high carotenoid content. It may be connected with differential resistance of plants to the mutagen (Privalov et al., 1986).

Table 8. Variations in carotenoid content in the fruit of the M1 seabuckthorn plants from seeds treated with ethylmethanesulphonate (EMS)

EMS Concentration (%)	*No of plants studied*	*Carotenoid content it in pulp of fresh fruit mg%)*		
		Means	*Min*	*Max*
Control	128	14.5±0.50	2.2	26.9
0.1	143	12.4±0.03**	6.5	23.8
0.5	115	11.0±0.30***	4.3	17.0
1.0	122	10.5±0.20***	7.3	16.8

Note: **The differences from control are significant at P = 0.99; *** at P = 0.999.

The effect of mutagens on the oil content

Seabuckthorn is characterized by the high variability in oil content in the fruit pulp. The variation range for oil content in individual Altai plants, is from 2.8-8.0% (Obodovskaya, 1957; Shishkina et al1985). In conditions of the West Pamirs, forms with oil content as high as 14.9% were found (Glazunova et al., 1983). Even within offspring grown from the seeds of a single maternal plant, oil content may differ three fold, from 1.7 to 5.1% (Table 9).

To study the plant variability, induced by the mutagens, seabuckthorn seeds were exposed to different doses of gamma rays. The experimental results indicated that, under the effect of ionizing irradiation, individual and group variability of plants, for fruit oil content widens. In the control, individual variability ranged from 2.8-8.4%, after the treatment of seeds with gamma-rays, variability widened from 1.1 to 9.3% (Privalov and Solonenko, 1977). The mean population value remained unaltered. Similar results were obtained, when the seeds were treated with chemical mutagen EMS (Privalov et al., 1986).

Table 9. Variations in oil content in seabuckthorn plants from seeds treated with EMS

EMS Concentration (%)	Population means and range (%)				Group means (%)			
	No. of plants studied	Means	Min	Max	No. of plants studied	Means for large fruit forms	No. of plants studied	Means for small fruit forms
Control	128	3.5±0.07	1.7	5.1	22	3.2±0.13	34	3.5±0.09
0.1	143	3.5±0.06	1.6	5.0	25	3.6±0.12*	29	3.6±0.12*
0.5	115	3.6±0.08	0.8	4.8	16	3.6±0.19*	20	3.2±0.13*
1.0	122	3.5±0.04	1.9	5.1	22	3.6±0.11*	19	3.5±0.15*

Note: *The differences from control are significant at P = 0.95.

From the data given in Table 9, it follows that, on the background, the mean population value unaffected by EMS, the group value for oil content in the large fruit forms tended to increase from 3.2% in control to 3.6%, depending on the concentrations of the mutagen. However, in contrast to gamma rays, there were no changes in the small fruit forms. This was presumably a manifestation of the specific effect of the chemical mutagen of EMS, compared to that of ionizing irradiation.

The effect of mutagens on sugar content

Individual variability range for the sugar content in wild seabuckthorn fruit during the period of their biological ripeness, varies from one distribution area to another, being from 0.6 to 1.2% in Azerbaidjan (Imamaliev, 1985) to 9.5% in the Altai territories (Kalinina and Panteleeva, 1978) and up to 12.4% in the Irkutsk region (Eliseev and Mishulina,1970). There appears to be a certain relationship between the climatic conditions of seabuckthorn and fruit sugar content.

A similar trend was observed when seabuckthorn seeds were exposed to the physical and chemical mutagens. Treatment of seabuckthorn seeds at high doses (from 30-100 kR) widened the individual viability range for fruit sugar content. The range was from 0.8 to 3.9% in the control and from 0.5 to 4.8% in the mutagen-treated seeds, with unaltered mean dry matter content at the control level (Privalov and Solonenko, 1977).

However, mean sugar content in the fruit increased from 2.2% in the control to 2.8% in the experimental variants. One of the reasons, why sugar content changed unidirectionally, was the mortality of the plants with low genetically predetermined sugar content in fruit at an early age, i.e. here one is dealing with the effects of selection of the more radio resistant forms, the characteristic is genetically correlated with future sugar content.

Widening of the individual and group variability in the fruit sugar content was also observed under the EMS effect. Individual variability range widened from 2.7 to 7.5% in the control to 1.8-9.3%, under the effect of different EMS doses. As in the case of fruit oil content, the changes in fruit sugar content were due to its increase in a small group of plants with the fruit of small size (Privalov et al., 1986).

The effect of mutagens on the ascorbic acid content

The wide individual variation in the ascorbic acid content is a characteristic feature of seabuckthorn fruit. This character varies from 8.0 to 1400 mg% and it has been related to various environmental factors, from the climate to location of fruit within the tree crown (Daems, 1963). In spite of the very wide environmental variability, ascorbic acid content is under certain genetic control. Analysis of segregation of ascorbic acid content in offspring of the same maternal plant demonstrated that this is, indeed, the case. Individual variability in ascorbic acid content in half- sib plants obtained from the “Ziryanka” variety in identical environmental conditions was in the range from 9 to 234 mg% and the mean value was 88 mg%. Treatment of “Ziryanka” seeds with gamma-irradiation at a dose of 10-25 kR certainly decreased the mean value of ascorbic acid from 129 mg% in the control to 92 mg% in experimental variants (Skuridin et al., 1998). Treatment of seeds with EMS induced no changes in the content of ascorbic acid in fruit (Privalov et al., 1986).

The effect of mutagens on fruit mass

Individual plant variability in fruit mass per 100 fruits in natural populations varies from 4.0- 100.0 g, which means that the larger fruit is 25 times heavier than the smallest. Our experimental data indicate that the range of the individual variability in fruit mass may be widened by mutagens. Thus, under the effect of treatment of seeds with gamma rays, the range of individual variability in the weight of 100 fruits increased from 20.0-46.6 g in the control to 14.5-48.7 g in the experimental series. The range became still wider under the effect of seed treatment with EMS. In the control, variability in the mass of 100 fruits was in the 22-58 range, and the range was 18-68 g after the treatment of seeds with EMS (Privalov et al., 1986).

INDUCED MUTATIONS IN SEABUCKTHORN BREEDING

In Russia, real seabuckthorn introduction in culture and breeding, began in the middle of 1930s as analytical breeding. It was carried out through selection, evaluation and vegetative propagation of the most valuable wild genotypes from Altai. The outstanding varieties like Novost, Altaya, Dar Katuni, Zolotoy pochatok, Vitaminnaya and Maslichnaya were created (Table 10). They did not loose their value even today. Presently the potentialities of analytical breeding seem to be exhausted.

Table 10. Some characteristics of new seabuckthorn varieties obtained from initial mutant forms “No 118” (var. Ziryanka) and “No 120”

Variety	*Fruit colour*	*Fruit weight (g/100)*	*Fruit pulp composition*				*Origin, specificities, uses*
			Sugars (%)	*Oil (%)*	*Ascorbic acid (mg/%)*	*Caro-tenoids (mg/%)*	
Ziryanka	Orange	65-70	5-6	3.6-5.5	129-190	13-15	Induced mutagenesis of wild Altai genotypes: Regularly high yield, high resistance to unfaourable factors. Universal variety.
Druzhina	Orange-red	70-80	5.6	3.9-5.6	167	15	Ziryanka (?) x No. 118/9 (?)* High resistance to frost, pests and diseases. Mechanical harvesting. Universal variety.
Zolotoy kaskad	Orange	74-90	4.6-6.1	2.6	97	10	Ziryanka (?) x No. 104 (?)* Regularly high yield. Universal variety. ”118/4” (?)*x No. 120/2 (?)**
Podruga	Orange	90-110	4.9-6.5	2.9	89	12	Extremely large fruits, good taste Dessert variety.
Krasny Fakel	Red	60-70	5.6	5.6-6.4	163	16-24	F, elite seedling of No. 120 form (free pollination) Late ripening. Universal variety.
Ivushka	Orange-red	70-80	3.6-5.4	4.8-5.8	123	16-19	Krasny Fakel (?) x No. 104 (?)* Very flexible shoots. Universal variety.
Ognistaya	Orange-red	60-70	5.0	5.1	73	24	Krasny Fakel (?) x No. 104 (?)* Thornless
Zamitsa	Orange-red	70-80	3.4	4.5	95	29	Krasny Fakel (?) x No. 104 (?)* Mechanical harvesting.

*F, seedling of “Ziryanka” (free pollination). **F1 seedling of “No. 120” (free pollination).

The second seabuckthorn breeding stage, called synthetic breeding, involves the hybridization of the parent genotypes, with a view to combine the most valuable characteristics into a common genotype. A number of new seabuckthorn varieties were developed and are being developed now. But it is not so effective as for annual crops, because of prolonged developmental cycle and comparatively small size of seabuckthorn population needed. Real progress in this speciescan be achieved by methods for increasing the frequency rate of valuable new genotype. This goal can be mainly reached by the experimental mutagenesis.

The manifestation of single gene mutation, in the somatic cells is dependent on the initial state of the allelic gene in the homologous chromosomes. The somatic mutation probability of a gene, i.e. its passage from dominant state (A) to recessive (a) and vice versa is about the same (A«a). However, its expression pattern is to a large extent dependent on the state of the internal (genotypic) and external environments. Dominant mutations are expressed both in the homozygous (AA) and in the heterozygous (Aa) condition, while the recessive is, as a rule, expressed only in the homozygous (aa). Because the heterozygous gene condition prevails in seabuckthorn, induced recessive mutations (Aa?aa) in the M, plants result in substitution of their phenotype by the alternative.

The genetics of particular seabuckthorn characters is not worked out, but one can be sure that most of valuable quantitative characteristics of this species are under multigenic control. This explains the high degree of environmental dependence of their expression. Mutant plants, occurring in nature and those produced by mutation breeding, can be useful for the applied breeding. As a rule, mutant plants carry one valuable character. Special efforts are needed to create a genotype combining needed character. The first mutant variety ‘Ziryanka’ is an example.

The pioneer works on the experimental mutagenesis in seabuckthorn were initiated by Privalov in 1959, at the Institute of Cytology and Genetics SD AN of the USSR. Seeds, collected from wild maternal plants of Altai population (the Chulishman river), were treated with gamma-irradiation at a dose of 15 kR (Privalov et al., 1971). The majority of the M, offspring showed the following undesirable dominant characters: thorny shoots, small fruit (11 to 42 g/100), short fruit-stems, dense distribution of fruit on the shoots, and fruit dehiscence during the picking. By exposure of seeds to irradiation and subsequent “dechimerisation” (cutting off shoots from 4-year old plants), forms with separate alternative characters were identified: thornless, with larger fruits, without dense distribution on the shoots, with longer fruit-stems. In the F, control offspring, such forms occurred at the extremely low frequencies, and they were weakly expressed. Although a few plants with two or three combined valuable characters, were found in the experimental material, none was found corresponding to the requirements of the new variety. Very low fruit yield was the main disadvantage of these best forms.

It appeared that a new variety can unlikely be developed by a single seed mutagenic treatment, even by the dechimerisation. Seeds, collected from various best mutant forms, were exposed to subsequent treatment with the chemical mutagen nitrosomethylurea (NMU, 0.01%). Mutant forms “No 118” and “No 120” were distinguished among the M, plants, obtained from the M, plants with larger fruits, longer fruit-stems and friable fruit distribution. They were of great interest to the breeders. The form “No 118” was distinguished by a set of valuable characters: long fruit- stems, solid large fruits, tearing without breaking, flexible branches carrying very few thorns, high growth rate and resistance to diseases. The results of competitive trials showed that mutant “No 118” was superior to the standard “Dar Katuni” variety. According to the average values for three years of trials, this form surpasses the standard. Its yield (11.4 t/ha) was by 75% more than that of standard variety. Fruit was larger; mean 100 fruit mass was 64 g, while it was 46 g in standard. Ascorbic acid content was two times higher. The form was named ‘Ziryanka’ variety.

In 1992, ‘Ziryanka’ was released as a commercial variety. The majority of the following varieties were created by the Institute of Cytology and Genetics of SD RAS in collaboration with Novosibirsk Zonal Fruit and Berry Growing Experimental Farm: “Druzhina”, “Podruga”, “Zolotoy kaskad”, “Ivushka”, “Ognistaya” and “Zamitsa”, bringing this variety’s genes (Shchapov and Belikh, 1999). It seems that this variety carries many latent mutant genes in the heterozygote and in “sleeping” state, including ability for parthenocarpy. The form “No. 120” had a number of valuable characteristics, including large fruit, good taste, high content of carotenoids and other vitamins. Thanks to good recessive genes in the homozygous state saturation by closely related crosses, this form became the parent of newest varieties “Krasni Fakel”, “Podruga”, “Ognistaya” and “Zamitsa” (Shchapov and Belikh, 1999). Some of these varieties can be harvested mechanically by shaking its shoots when frozen in winter (Shchapov et al., 1998).

Thus, for obtaining the promising mutant forms of seabuckthorn, by the method of experimental mutagenesis, we had double-treated seeds with different mutagens. These treatments were associated with parallel selection of the best primary forms for the subsequent mutagen treatment. This increased the probability of obtaining forms, offering promise in breeding. The experience of breeders shows that both male and female mutant forms are equally valuable in breeding. The data of Table 10 shows that pollen of male mutant plants carries valuable characteristics to improve the female parent genotype. The comparative characteristics of the new varieties derived from mutants “No 118” and “No 120” through selection are illustrated in Table 10. All new varieties are almost thornless, have various times of fruit ripening and high resistance to various kinds of the unfavourable factors. The main results of seabuckthorn mutation breeding obtained by researchers from various stations of Russia are shown in Table 11.

Table 11. The main results of mutational breeding in seabuckthorn

Breeding station	*Initial breeding material*	*Results of mutational breeding*	*Mutagen (s) applied**	*References*
1. Institute of Gytology and Genetics, Novosibirsk, (Russia)	Wild genotypes from Altai (Chulishman river) populations	Var. Ziryanka, Druzhina, Zolotoy kaskad, Krasny fakel, Ivushka, Ognistaya, Zamitsa, “N0.H8-P” (parthenocarpic)	Gamma-irradiation (15 kR) in M1 and NMM in M2	Genetika - selektsii rasteny, 1983; Shchapov, Kreymer, 1998; Shchapov, Belikh, 1999
2. Nizhny Novgorod State Agricultural Academy, N. Novgorod (Russia)	Var. Novost’ Altaya, Dar Katuni, Zolotoy pochatok, Maslichnaya, Shcherbinki-1, Shcherbinki-2, Shcherbinki-3	Var. Pamyat’ Rapoporta, Urozhainaya, Novinkovskaya, Nizhegorodskaya sladkaya, Zarevo, Maria, Duet	DMS, El, NMU, NDMU, NEU and various doses of gamma-irradiation	Eliseev, Mishulina, 1974 Eliseev, 1985b Eliseev et al., 1988 Fefelov, 1998
3. Michurin Research Institute of Horticulture, Michurinsk (Russia)	Var. Vitaminnaya	Var. Pamyat’ Indiri Gandy, Michurinsk-350, Ulibka, Vostochnaya krasavitsa, Solnechy Dozhd’, Gordost’ CGL	Gamma-irradiation (0.15-5.0 kR)	Zhukov, Mokrousova, 1985; 1986; 1987; 1993
4. The Buryat Fruit and Berry Experimental Station, Ulan-Ude (Russia)	Wild elite forms of Buryat, Tuva, Mongolia populations, range of local varieties	Var. Zarya Dabat and range of prospective elite forms	Gamma- i rradi ation (0.5 kR)	Myahanova, 1998
5. Central Botanical Garden of NAS, Minsk (Bielarus’)	Var. Novost’ Altaya, Vitaminnaya, Obskaya, Prevoskhodnaya, Yantarnaya	Three prospective elite forms and about 200 ordinary mutants	DB, DES, DMS, El, NEU, NMU	Garanovitch, 1991 Garanovitch, Shpital’naya, 1993
6. Northern Research Institute of forestry, Arkhangelsk (Russia)	Wild forms from Baltic region and Altai (Katun’ river)	A range of prospective forms	DAB, NEM, NMM	Nilov, 1987 Demidova, Nilov, 1988
7. Primorskaya Fruit and Berry Farm, Vladivostok (Russia)	Var. Chuiskaya, Velikan, Prevoskhodnaya	Five prospective elite forms and more than 400 mutagen-treated seedlings	Gamma-irradiation (5-10 kR)	Oksenyk, 1993; 1998

*DB = Diazobenzene, DES = Diethylsulphate, DMS = Dimethylsulphate, El = Ethyleneimine, EMS= Ethylmethanesulphonate, NDMU = Nithrosodimethylurea, NEU = Nithrosothylurea, and NMU = Nithrosomethylurea.

CONCLUSIONS

In Russia, work on breeding of seabuckthorn had started few decades ago. Since then, more than 50 seabuckthorn varieties have been developed in Russia. The results of mutation breeding are also noteworthy. Experimental mutagenesis can greatly facilitate breeding of new varieties, thanks to the induction of valuable characters that rarely emerge in natural conditions. Based on the analysis and generalisation of experience with mutagen application, the inferences are:

1. General patterns of ionizing irradiation and chemical action were observed in seabuckthorn. Mutagen treatment widens the genetic variability that is of great importance for breeding the new varieties.

2. Most varieties were obtained by mutagen application at the relatively low doses. This indicates that low doses meet more the requirements of breeding.

3. Mutagen can have long-term effects. Mutations can be revealed after many years by the vegetative propagation.

4. Deviations from the main values of characteristics (certain variability) in mutagen-treated population, indicate that somatic cell selection operates during the development of seabuckthorn plants and/or that there are prolonged (long-term) effects of mutagens.

REFERENCES

1. Belikova, A.F. 1982. Peculiarities of mutagenic stimulation of Siberian larch. Uluchshenye kul'turnikh rasteny i khimichesky Mutagenez, p. 225-231, Moscow. (In Russian)

2. Daems, W.F. 1963. De fytochemie Van de duindoorbessen. Chem. Courant. 62: 808-813.

3. Demidova, N.A. & Nilov, V.N. 1988. The use of breeding methods in seabuckthorn introduction in the European north of the USSR. Biologiya, selektsiya i agrotekhnika oblepikhi, p. 51-63, Gor'ky. (In Russian)

4. Eliseev, I.P. 1985 a. Some theoretical aspects and prospects for breeding of seabuckthorn in the European part in the USSR. Biologicheskie aspekti introduktsii, selektsii i agrotechniki oblepikhi, p. 3-24, Gor'ky. (In Russian)

5. Eliseev, I.P. 1985 b. Breeding of seabuckthorn. Oblepikha. p. 74-87, Moscow. (In Russian)

6. Eliseev, I.P. & Mishulina, I. A. 1970. Biological specialities of East-Sibirian population of seabuckthorn and breeding of high productivity forms. Oblepikha v kulture, p. 51-54, Barnaul. (In Russian)

7. Eliseev, I.P. & Mishulina, I.A. 1974. Sensitivity of seabuckthorn seeds to gamma-irradiation. Plodovo-yagodnie kul'turi, Gor'ky 77: 107-109. (In Russian)

8. Eliseev, I.P., Fefelov, V.A. & Mishulina, I.A. 1976. Biological active substances in North Caucasus and Middle Asia seabuckthorn fruits. Plodovie iyagodnie kul'turi, Gor'ky 100: 42-45. (In Russian)

9. Eliseev, I.P., Mishulina, I.A. & Kreknina L.I. 1982. The results of application of chemical mutagens in seabuckthorn selection. Uluchshenie kul'turnikh rasteny i khimichesky mutagenez, p. 215-219, Moscow. (In Russian)

10. Eliseev, I.P., Mishulina, I.A. & Kreknina L.I. 1988. The results of application of chemical mutagenesis in seabuckthorn selection. Biologiya, selektsiya i agrotekhnika oblepikhi, p. 4-18, Gor'ky. (In Russian)

11. Fefelov, V.A. 1998. Summary and selection purpose of Hippophae rhamnoides in the Volgo-Vyatka region. In: Proceedings of International Symposium on Seabuckthorn, p. 149-150, Novosibirsk.

12. Garanovitch, I.M. 1991. Introduction, breeding and cultivation of seabuckthorn in BSSR. Novoe v biologii, khimii i farmakologii oblepikhi, p. 16-23, Novosibirsk. (In Russian)

13. Garanovitch, I.M. & Shpital'naya, TV. 1993. Seabuckthorn selection in Bielarus'. In: Proceedings of International Symposium on Seabuckthorn, p. 22-24, Novosibirsk. (In Russian)

14. Genetika Selektsii Rasteny. 1983. p. 21, Novosibirsk. (In Russian)

15. Glazunova, E.M., Gachechiladze, N.D., Bondar', V.V. & Korzinnikov, Yu.S. 1983. Biochimical study of seabuckthorn in West Pamir mountains. Biologiya, khimiya i pharmakologiya oblepikhi / p. 33-36, Novosibirsk. (In Russian)

16. Imamaliev, G.N. 1983. Seabuckthorn in Azerbaijan. Biologiya, khimiya i pharmakologiya oblepikhi, p. 16-19, Novosibirsk. (In Russian)

17. Kalinina, I.P. & Panteleeva, E.I. 1978. Seabuckthorn selection in Altai. Oblepikha, p. 56-79, Moscow. (In Russian)

18. Korzinnikov, Yu.S., Krimskaya, N.B. & Gachechiladze, N.D. 1983. Biological and morfological features of seabuckthorn in West Pamir. Biologiya, khimiya i pharmakologiya oblepikhi, p. 19-24, Novosibirsk. (In Russian)

19. Muraviova, D.A. & Lagazidze D.S. 1985. Resources of seabuckthorn in North Caucasus and Georgian republic. Biologicheskie aspekti introduktsii, selektsii i agrotechniki oblepikhi, p. 139-141, Gor'ky. (In Russian)

20. Lbova, M.I. 1998. Change of the ratio of sexual types in posterity of the variety Ziryanka. In: Proceedings of International Symposium on Seabuckthorn, p. 148-149, Novosibirsk.

21. Manual on Mutation Breeding. Vienna, IAEA, 1977. Technical Reports Ser. Sec. Ed. No 119. 288p.

22. Mutation Breeding Newsletter. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. Vienna, IAEA, 1989, No 34, p. 1.

23. Myahanova, N.T. 1998. The results of seabuckthorn selection in Buryatia. In: Proceedings of International Symposium on Seabuckthorn, p. 146-147, Novosibirsk.

24. Nilov, V.N. 1987. Introduction of seabuckthorn on European north of the USSR and its selection fund in the dendrology garden of Arkhangel'sk Institute of forestry. Biologiya, selektsiya i agrotekhnika plodovikh iyagodnikh kultur, p. 34-39, Gor'ky. (In Russian)

25. Obodovskaya, D.A. 1957. Seabuckthorn as a raw material for vitamin industry. Moscow. (In Russian)

26. Oksenyk, Y.F. 1993. Selection, variety testing ant specification of seabuckthorn culture in monsoon climate of sea country region. In: Proceedings of International Symposium on Seabuckthorn, p. 41-42, Novosibirsk. (In Russian)

27. Oksenyk, Y.F. 1998. Selection of initial forms and ways of development acceleration of the new seabuckthorn varieties in conditions of climate of the Far East of Russia. In: Proceedings of International Symposium on Seabuckthorn, p. 156-157, Novosibirsk.

28. Potapov, S.P., Borodachev, M.N., Nikitenko, S.S., & Borodacheva, L.D. 1978. The variability of seabuckthorn under the action of chemical mutagens. Khimichesky mutagenez i hybridizatsiya, p. 154-157, Moscow. (In Russian)

29. Privalov, G.F. 1966. Induced mutations in the germinative and vegetative organs of woody plants of the first generation. Eksperimental 'ny mutagenez sel skokhozyaistvennikh rastenii i ego ispol 'zovanie v selektsii, p. 249- 254, Moscow. (In Russian)

30. Privalov, G.F. 1968. Study of experimental mutagenesis in woody plants. Genetika 6: 144-157. (In Russian)

31. Privalov. G.F. & Shmeleva, Ju.F. 1971. Radiosensitivity of seabuckthorn seeds. Radiobiologiya 11: 421-425. (In Russian)

32. Privalov, G.F., Solonenko, L.P. & Trofimova, O.S. 1971. Variability in the chemical composition of Hippophae rhamnoides L. in consequence of ionising radiation of seeds in stimulating doses. Izvestiya SO AN SSSR, Ser.biol.nauk 10: 72-75. (In Russian)

33. Privalov, G.F. & Solonenko, L.P. 1977. Ionising radiation as a factor of selection in seabuckthorn. Genetika 13: 2045-2046. (In Russian)

34. Privalov, G.F., Skuridin, G.M. & Solonenko, L.P. 1983. The effect of mutagens and gibberellin treatment on chemical composition of seabuckthorn fruits in Mr Biologiya, khimiya i pharmakologiya oblepikhi, p. 49-56, Novosibirsk. (In Russian)

35. Privalov, G.F., Skuridin, G.M., & Solonenko, L.P. 1986. Individual and group variability in the chenmical composition of EMS-induced M| contrasting seabuckthorn forms. Genetika 22: 2477-2484. (In Russian)

36. Shchapov, N.S. 1979. Karyology of Hippophae rhamnoides L. Tsitologia and Genetika 13: 45-47. (In Russian)

37. Shchapov, N.S. 1999. Cytological and genetical methods in seabuckthorn breeding. Oblepikha v lesostepi Priob'ya, p. 29-49, Novosibirsk. (In Russian)

38. Shchapov, N.S. & Belikh, A.M. 1999. Seabuckthorn varieties selected by the Institute of Cytology an Genetics of SD RAN and Novosibirsk Zonal Fruit and Berry Growing Experimental Station. Oblepikha v lesostepi Priob'ya, p. 50-55, Novosibirsk. (In Russian)

39. Shchapov, N.S., Belikh, A.M. & Goncharova, L.A. 1998. Red-fruited seabuckthorn varieties perspective for mechanization of fruit harvesting. In: Proceedings of International Symposium on Seabuckthorn, p. 197, Novosibirsk.

40. Shchapov, N.S. & Kreimer, V.K. 1998. The 118-P-A partenocarpic form of seabuckthorn. In: Proceedings of International Symposium on Seabuckthorn, p. 152-153, Novosibirsk.

41. Shishkina, E.E., Loskutova, G.A. & Arkhipova, T.N. 1985. Biochemical characteristics of seabuckthorn varieties. Biologicheskie aspekti introduktsii, selektsii i agrotechniki oblepikhi, p. 117-128, Gor'ky. (In Russian)

42. Skuridin, G.M., Lbova, M.I., Privalov, G.F. & Solonenko, L.P. 1998. The remote influence of gamma-irradiation and phytohormones on chemical stricture of seabuckthorn fruits. In: Proceedings of International Symposium on Seabuckthorn, p. 214-215, Novosibirsk.

43. Vavilov, N.I. 1922. The law of homologous series in variation. J. Genetics 21: 47-49.

44. Zhukov, O.S. & Mokrousova, G.I. 1985. The new prospective seabuckthorn forms for Central Chernozem Area. Phiziologiya i biokhimiya kulturnikh rasteny, p. 92-96, Gor'ky. (In Russian)

45. Zhukov, O.S. & Mokrousova, G.I. 1986. The improvement of biochemical composition of seabuckthorn by radiation mutagenesis. Biologiya, khimiya, introduktsiya i selektsiya oblepikhi, p. 67-68, Gor'ky. (In Russian)

46. Zhukov, O.S. & Mokrousova, G.I. 1987. The new prospective seabuckthorn varieties for Middle Strip of the USSR. Issledovaniya oblepikhi i oblepikhovogo masla, p. 5, Novosibirsk. (In Russian)

47. Zhukov, O.S. & Mokrousova, G.I. 1993. Production seabuckthorn forms promising for the Central chernozem zone of Russia. In: Proceedings of International Symposium on Seabuckthorn, p. 203-205, Novosibirsk.