Apomixis Manifestation in Seabuckthorn (*Hippophae rhamnoides* L.)

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ABSTRACT

The visual examination of the metaphasic plates in the root meristems of seabuckthorn (Hippophae rhamnoides L.) showed the presence of solitary cells with a chromosome number, non-typical for the species (2n = 18, 20, 21, 30, 48). Based on the studies of several researches about the prevalence of an aneusomy phenomenon (inconstancy of a chromosome number in the cells and tissues within a plant) among the apomicts, it is possible to suppose that aneusomy of the seabuckthorn meristematic cells, observed both in the present investigation and the assays of some other workers, can be related with seabuckthorn ability to apomixis. Through the analysis, it was observed that male samples with low pollen viability and fertility, most of the pollen grains have no nucleus at all or have one nucleus only. It is a non-typical phenomenon for the species.

Key words: Seabuckthorn (Hippophae rhamnoides L.), chromosome numbers, pollens and apomixes.

INTRODUCTION

Apomixis, as peculiar form of sexual reproduction in the flowering plants, has been and remains a matter of contention and discussions from the point of determination of its evolutionary significance. With respect to sexual reproduction, apomixis is considered to be a secondary phenomenon. Most frequently apomixis is observed in the younger and more progressive in an evolutionary sense, as manifested in families and genera like Taraxacum, Alchemilla, Hieracium, Fragaria, Poa and Rubus etc.

Seabuckthorn was not included in the Khokhlov's lists of the apomictic species (1967), but in the literatures related to biology of seabuckthorn, some investigators reported about its features usual for apomictic species. The monograph by Servettaz (1909) devoted to the Elaeagnaceae family, has the same remarks concerning anomalies of micro-and megasporogenesis in seabuckthorn. According to the findings of Servettaz, the second meiotic division takes place in a micro-polar cell, only, whereas a chalazal cell becomes eight-nucleic embryo sac, without any division of the cell. For the first time, manifestations of the features, that characterize apomictic appearance of parthenocarpic fruits and seeds without pollination, polyembryony in seabuckthorn were first noted by Eliseev (1979). Later on, this phenomenon was mentioned in papers of several other authors (Nikitin, 1989; Zhukov and Mokroussova, 1993). Apomicts undergo the phenomenon of mixoploidy (aneuploidy) more often than amphimicts do. In the reports of Aratyan (1937, 1940), Eliseev and Mishulina (1974) and Borodina (1982), there are findings on mixoploidy appearance in meristematic cells of seabuckthorn. It is a characteristic feature of apomicts. It is as much as discussed question of importance not only for theoretical investigations, but also for practical goals of seabuckthorn industrial cultivation. We have been performing the purposeful studies of apomixis manifestation in seabuckthorn over a period of several years.

MATERIALS AND METHODS

As the subject of our studies, we selected the female plants of seabuckthorn from several varieties of Altay selections (i.e. Samorodok, Yantamaya, Obilnaya, Chujskaya), from two varieties of Moscow State University Botanical Garden selections (Trofimovskaya-35 and Trofimovskaya-7), as well as from two varieties (Scherbinki-1, Nizhegorodskaya Sladkaya) and two elite forms (ShNMM-8, DMS-5) of Nizhny Novgorod Agricultural Academy's selection. Besides, female seedlings from Baltic population were also included in our study. Both as a control (with open pollination) and test (without pollination), variants were put on the neighboring branches of the same height. Isolation of pistillate flowers was performed by a conventional technique several days before the beginning of florescence and coarse

Calico bags were used as the isolators. Then, the seeds, which had been obtained through open pollination and without pollination, were sprouted with subsequent determinations of ploidy in meristematic cells of the rootlets, with propion-lackmoid being used as reagent (Rudenko and Dudukal, 1972).

Khokhlov et al. (1968) have noted that species, which are prone to apomixis, are characterised by a higher degree of pollen deficiency (failure) and they elaborated amorphological method of natural apomictic form searching, which is based on the estimate of pollen deficiency. Relying on such data, we have undertaken a cytogenetic and histo-chemical analysis of pollen from some perspective male forms, taken in the hybrid families of the gene pool belonging to the Department of Fruit and Berry Crops Selection and Introduction (Nizhny Novgorod State Agricultural Academy). Potential pollen viability was estimated using simultaneously several histo- chemical techniques, i.e. acetocarminic one and Isatinic test (for proline content). Pollen fertility was determined by a direct method with pollen germination on the Trankovsky substrate 1% Agar-Agar, 0.0001% Acid Boric and 0.0003% CaCl0. A number of the nuclei in the pollen grains were determined by propion-lackmoid staining of a pollen sample during 30-40 minutes.

RESULTS AND DISCUSSION

It was found through our studies, that a number of fruits with normal plump seeds in the isolated branches of the seabuckthorn female plants did not differ, essentially, from the control variant (Table 1). And plausible diminution in the number of fruits at the fruit buds was observed only in Samorodok and Trofimovskaya-35 varieties. Besides, significant differences in the seed and fruit weight between control and trial variants for the majority of varieties were not registered, either (Table 1).

Table 1. Number of fruits in the fruit buds, the weight of seabuckthorn fruits and seeds obtained through open pollination (control) and without pollination (test)

Varieties	Number of fruits in the fruit buds (pcs)		Weight of 100 seeds (g)		Weight of 100 fruits (g)	
Altai selection varieties						
	control	test	control	test	control	test
Samorodok	3.4	2.6*	18.7	20.3	61.1	69.5*
Yantamaya	3.4	3.4	18.7	19.5	65.9	67.5
Obilnaya	2.1	2.3	19.5	20.3	75.6	73.5
Chujskaya	3.5	3.4	21.4	22.6	85.4	85.9
MSU - Botanical Garden selection varieties						
Trofimovskaya-35	6.8	3.3*	17.2	17.9	62.6	68.6
Trofimovskaya-7	3.8	3.2	18.8	17.8	66.0	63.9
Nizhny Novgorod Agricultural Academy selection varieties and perspective forms						
Scherbinki-1	2.6	2.1	18.0	18.6	66.5	68.6
Nizhegorodskaya sladkaya	2.1	3.4	18.8	17.8	66.0	63.9
ShNMM-8	2.4	2.4	16.4	18.5*	50.4	54.5
DMS-5	1.6	1.5	22.7	23.7	75.2	77.5
Baltic population	4.6	4.4	15.4	15.5	29.0	29.4

Note: *Differences are significant at $p < 0.05$.

The seeds of the trial variant (condition without pollination) possessed good sprouting ability. The sprouts from seeds of the apomictic, in our opinion, origin did not differ in their growth characteristics from those of the control variant, and their ploidy was typical for this species (2n = 24). The visual examination of the metaphasic plates in the root meristems showed the presence of solitary cells with a chromosome number, non-typical for the species (2n = 18, 20, 21, 30, 48) (Table 2). Some authors (Araratyan, 1937, 1940; Eliseev and Mishulina, 1974; Borodina, 1976) had also mentioned a mixoploidy of the seabuckthorn somatic cells. Based on the opinion of several researches about the prevalence of an aneusomy phenomenon (inconstancy of a chromosome number in the cells and tissues within a plant) among the apomicts, it is possible to suppose that aneusomy of the seabuckthorn meristematic cells, observed both in our investigations and in the assays of some other workers, can be related with seabuckthorn ability to apomixis.

Table 2. Chromosome numbers of cells from root meristems of seabuckthorn sprouts

Varieties	Variant	Total	Amount of metaphasic plates with chromosome number (2n) %						
			12	18	20	21	24	30	48
Scherbinki-1	Test		3.3	3.3	8.2	4.9	60.6	13.1	6.6
	Control	28	-	7.1	10.7	7.1	64.3	7.1	3.6
Trofimovskaya-35	Test	11	-	-	18.2	9.1	63.6	9.1	-
	Control	13	-	7.7	7.7	-	76.9	7.7	-

Note: The seed sprouted were obtained through open pollination (control) and without pollination.

Results of pollen cytogenetic analysis are given in Table 3. Through the analysis, it was observed that male samples with low pollen viability and fertility, most of the pollen grains have no nucleus at all or have one nucleus only. It is a non-typical phenomenon for the species. Estimation of pollen fertility by the way of germination on the Trankovsky substrate has shown that more than 50 % of fertile pollen grains were revealed in only 5 of 25 samples studied.

In the course of the studies performed, we revealed a high positive correlation (r = 0.9) between the proline content in the pollen grains and fertility. The viable pollen grains were rich in proline (Table 3). In an experiment carried out in the spring of 1998, a pollen analysis of 20 male forms has revealed a lower level of pollen fertility, as only 5 of freshly investigated samples had more than 50 percent of viable pollen grains. In our opinion, such a lower degree of pollen viability can serve as an indirect evidence for seabuckthorn's ability for apomictic reproduction.

Table 3. A nuclei amount and viability of mature pollen in the male forms from the seabuckthorn hybrid families

Male forms	Number of nuclei in the pollen grains (%)			Viability of pollen (%) estimated by different techniques		
	One nucleus	two nuclei	non nucleated	acetocarminic technique	proline test	sprouting on the substrate
1/95	11.5 ± 1.6	14.7 ±3.0	73.7 ±4.1	79.6 ±4.1	4.0 ±1.3	14.5 ± 1.9
2/95	21.3 ± 1.4	31.2 ±2.3	40.6 ± 1.9	96.0 ± 1.7	45.4 ±3.1	39.9 ±3.5
3/95	30.6 ± 5.3	34.9 ± 4.5	31.3 ±3.2	81.9 ±3.6	60.7 ± 4.6	40.0 ± 2.8
4/95	23.9 ± 3.4	17.1 ± 1.6	59.0 ± 3.6	82.7 ± 4.4	23.7 ± 3.7	22.7 ± 2.2
6/95	29.1 ±2.9	45.1 ±3.4	24.6 ±4.1	88.1 ±2.4	49.6 ± 5.3	49.9 ± 3.2
7/95	20.2 ± 4.7	46.4 ± 4.6	20.3 ± 5.6	88.8 ± 3.4	64.2 ± 6.6	45.5 ± 4.2
8/95	30.0 ± 1.6	47.6 ±2.1	22.4± 2.8	90.3 ± 1.7	68.0 ±5.1	70.0 ± 2.8
9/95	19.3 ±2.1	58.0 ±2.4	22.7 ± 0.9	96.5 ± 1.1	70.7 ± 3.8	57.6 ± 1.6
10/95	28.2 ± 6.7	34.1 ±2.7	37.8 ± 6.0	96.2 ± 1.5	50.7 ±3.6	33.5 ± 2.4
12/95	36.8 ± 2.2	24.3 ± 3.4	39.9 ±2.1	89.2 ± 4.4	36.5 ± 3.2	31.5 ± 1.4
13/95	23.2 ±4.8	50.5 ± 3.9	26.3 ± 3.5	86.7 ± 2.3	49.3 ± 3.6	38.3 ± 1.3
14/95	31.2 ± 1.2	29.1 ±3.2	37.3 ± 2.6	93.9 ± 1.8	50.7 ± 2.7	33.7 ±2.4
15/95	35.6 ±2.0	33.4 ± 2.2	30.0 ± 2.3	94.5 ± 2.0	75.9 ± 3.9	57.8 ±5.6
16/95	22.2 ± 3.4	29.6 ± 2.0	48.2 ± 2.7	91.4 ± 1.4	35.3 ±2.2	18.2 ±2.5
17/95	41.2 ±2.7	42.7 ± 3.2	13.9 ± 1.6	96.2 ± 1.3	86.9 ± 2.9	84.6 ± 2.4
18/95	39.2 ± 2.7	19.4 ±3.6	41.4 ± 1.5	77.4 ± 2.4	35.7 ± 1.2	29.4 ± 2.3
19/95	30.5 ± 4.4	37.6 ±3.8	31.9 ±2.7	95.6 ± 1.1	64.7 ± 6.6	61.4 ±5.3
20/95	24.6 ± 4.6	17.3 ± 1.7	58.1 ±5.8	80.7 ± 2.8	16.0 ±3.2	18.3 ± 1.7
21/95	39.1 ±3.9	29.9 ± 1.9	31.0 ±2.7	94.2 ± 0.6	49.4 ± 2.3	35.9 ±3.2
22/95	22.3 ± 2.4	20.5 ± 1.7	40.0 ± 3.6	88.2 ± 1.9	43.8 ± 2.5	41.0 ±3.4
23/95	29.0 ± 4.8	27.1 ± 1.7	43.8 ± 4.2	85.8 ± 2.0	42.5 ± 2.7	36.6 ± 1.3
24/95	32.8 ±5.6	43.5 ± 4.4	19.7 ±4.6	83.6 ±2.5	53.8 ±7.7	34.3 ± 1.8
25/95	24.4 ±5.1	24.2 ± 1.6	514 ±4.6	89.3 ±2.1	32.2 ± 1.7	31.5 ±2.5
26/95	17.0 ±3.6	21.8 ±0.8	61,2 ±4.2	89.9 ± 2.9	26.3 ± 1.3	23.9 ±3.1
27/95	16.2 ±3.4	20.2 ± 1.9	63.6 ±2.6	72.1 ±5.3	11.7 ± 1.8	18.4 ± 1.8

Thus, some anomalies noted previously, by several researchers in megasporogenesis and microsporogenesis, polyembryony, ability for autonomous and induced apomixis as well as our own data newly obtained after experimental investigations with the isolation of the pistillate flowers, together with examinations of pollen from the male forms are indicative of seabuckthorn ability for the apomixis. Of course, the nature of this phenomenon is extremely difficult and it needs further investigations in this direction.

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