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Mutagenic treatment of pollen to obtain an inbred generation from self-incompatible cultivars of apple (*Malus domestica* Borkh.) and pear (*Pyrus communis* L.)

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Abstract.

Aims. The information analysis on the gene expression of self-incompatibility in apple (*Malus domestica* Borkh.) and pear (*Pyrus communis* L.) cultivars and the generalization with our research results on overcoming self-incompatibility by pollen mutagenic treatments were determined to be the aim of this article. ***Methods***. The studies of the controlled geitonogamy were conducted at the orchards of the National Dendrological Park "Sofiyivka" of the NAS of Ukraine (NDP "Sofiyivka") and the Uman National University of Horticulture (UNUH). The experiments were performed on 10- to 20-year-old apple trees Golden Delicious', 'Zymove lymonne', 'Cortland' and 'Slava Peremozhcyam', as well as pear trees 'Bere Desiatova' and 'Clapp's Favorite'. Castrated flowers of these cultivars were pollinated with pollen from other flowers of the same tree, i.e. geitonogamy was performed (one of the variants of inbred pollination), and also a number of variants of geitonogamy by mature pollen preliminarily treated with gamma rays in doses of 5; 20 and 50 gray (Gy) and chemical mutagens vapours: nitrosodimethylurea (NDMU—50 mg per desiccator; dimethyl sulfate (DMS) and ethylene oxide (EO)—both 12 drops per ten-litre sealed desiccators. The statistical analyses were carried out using the methods of Ronald Fisher. ***Results.*** The efficiency of the mutagens used for increasing the percentage of fruit set and seeds per fruit was confirmed in most variants of the experiments with apple (*M. domestica*) and pear (*P. communis*). We assumed that after geitonogamous pollination by many mutagenized pollen grains with mutant and non-mutant alleles of the *S*-gene, the inhibition in the stigma and/or in the ovary mainly may show for pollen grains without mutant alleles of the *S*-gene. Analysis of the fruit set showed that in all variants with mutagens, the fruit set and also the number of seeds per fruit significantly exceeded variants of "geitonogamy without mutagens". It also was revealed that the average fruit set and the average number of seeds per fruit in apple cultivars were much higher than in pear cultivars. ***Conclusions*** Studies have shown that treatment of apple and pear pollen with mutagens promotes fruit setting and the number of seeds per fruit in geitonogamous pollination, possibly due to the natural selection of mutant microspores by the egg apparatus of the female gametophyte and the germination inhibition of non-mutant pollen. The technique of geitonogamous pollination by pollen pre-treated with gamma rays and chemical mutagens for obtaining an inbred generation can be recommended for apple and pear breeding, and also deserves to be studied in other cultivated plants with self-incompatibility genes.

Key words: apple tree, hermaphrodite plants, gametophytic self-incompatibility, geitonogamy, gene expression, pear tree, artificial pollination, pomaceous, *S*-alleles.

Обробка пилку мутагенами для отримання інбредного покоління від самонесумісних сортів яблуні (*Malus domestica* Borkh.) й груші (*Pyrus communis* L.)

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Реферат.

Мета. Аналіз інформації щодо експресії генів самонесумісності у сортів яблуні (*Malus domestica* Borkh.) й груші (*Pyrus communis* L.) та узагальнення її з результатами наших досліджень можливостей використання мутагенної обробки пилку для подолання самонесумісності було визначено метою виконаної роботи. ***Матеріали і методи***. Дослідження контрольованої гейтоногамії проводили в плодових садах Національного дендрологічного парку «Софіївка» НАН України (НДП «Софіївка») та Уманського національного університету садівництва (УНУС) на 10–20-річних деревах яблуні сортів ‘Голден Делішес’, ‘Зимове лимонне’, ‘Кортланд’ і ‘Слава Переможцям’, а також сортів груші ‘Бере Десятова’ й ‘Улюблена Клаппа’. Кастровані квітки цих сортів запилювали пилком з інших квіток того самого дерева, тобто проводили гейтоногамію (один із варіантів інбредного запилення), а також виконували ряд варіантів гейтоногамії зрілим пилком, попередньо обробленим гамма-променями в дозах 5; 20 і 50 грей (Гр) та парами хімічних мутагенів: нітрозодиметилсечовини (NDMU — 50 мг на ексикатор; диметилсульфату (DMS) і етиленоксид (EO) — обидва по 12 крапель на десятилітровий герметичний ексикатор. Статистичний аналіз проводили за Рональдом Фішером. ***Результати та обговорення***. Ефективність використання мутагенів для підвищення відсотка зав’язування плодів і насіння на плід підтвердилася в більшості варіантів дослідів з яблунею (*M. domestica*) і грушею (*P. communis*). Це дало підстави припустити, що за гейтоногамного запилення багатьма обробленими мутагенами пилковими зернами з мутантними і немутантними алелями *S*-гену інгібування на приймочці та/або в зав’язі може проявлятися переважно для пилкових зерен без мутантних алелей *S*-гену. Аналіз зав'язування плодів показав, що у всіх варіантах з мутагенами зав'язування, а також кількість насінин у плоді значно перевищували варіанти «гейтоногамії без мутагенів». Також виявлено, що середня зав’язуваність плодів і середня кількість насінин на плід у сортів яблуні були значно вищими, ніж у сортів груші. ***Висновки.*** Дослідження засвідчили, що обробка пилку яблуні та груші мутагенами сприяла зав’язуванню плодів і збільшенню кількості насінин на плід при гейтоногамному запиленні. можливо, внаслідок природного добору мутантних мікроспор апаратом яйцеклітини жіночого гаметофіта й інгібування проростання немутантного пилку. Техніка гейтоногамного запилення попередньо обробленим гамма-променями та хімічними мутагенами пилком для отримання інбредного покоління, може бути рекомендована для селекції яблуні та груші, а також заслуговує на вивчення на інших культивованих рослинах з генами самонесумісності.

Ключові слова: яблуня, гермафродитні рослини, гаметофітна самонесумісність, гейтоногамія, експресія генів, груша, штучне запилення, зерняткові, *S*-алелі.

Introduction. The apple (*Malus* Mill.) and pear (*Pyrus* L.) are pomaceous fruit tree genera of the *Rosaceae* family (Bell & Itai, 2011; Engler, 1903; Ignatov & Bodishevskaya, 2011; Quinet & Wesel, 2019). Both genera belong to the subtribe *Malinae* Rev., tribe *Maleae* Small, of the big subfamily *Amygdaloideae* Arn., which combines the former subfamilies *Amygdaloideae, Spiraeoideae* C. Agardh and *Pomoideae* Focke, =*Maloideae* Weber (Li et al., 2019; Opalko et al., 2016; Postman, 2019; Turland et al., 2018).

In nature, *Malus* and *Pyrus* plants are allogamous and entomophilous, but their cultivars reproduce by the diverse methods of asexual vegetative propagation, usually by grafting, mainly by budding (Chandler, 1957). Therefore, apple and pear cultivars in orchards consist of clones, i.e., genetically identical individuals (ramets). The vegetative propagation allows the elite commercial cultivars to remain for many years in industrial orchards (Migicovsky et al., 2021). The majority of *Malus* and *Pyrus* cultivars are diploid (2*n*=34). However, some triploids and tetraploids exist (Bell & Itai, 2011; Kellerhals, 2009; Phillips et al., 2016).

The scientific name of apple cultivars listed in the State Register of plant varieties suitable for dissemination in Ukraine in 2021 is *Malus domestica* Borkh., with synonyms *Purus malus* L. and *Malus communus* Lam. (Apple, 2021); respectively, of pear cultivars—*Pyrus communis* L., with synonyms *Pyrus pyraster* Borkh., *Pyrus sativa* Koch, *Pyrus achras* Gaertn., and *Pyrus domestica* Medic. (Pear, 2021).

The production efficiency of apple and pear fruits, as well as horticulture in general, is determined by cultivar potentials and technologies. That is, cultivars can be considered one of the principal means of apple and pear fruit production. Therefore, the crop capacity and adaptability of cultivars, as well as any other means of production, need to be continuously improved. Different methods of breeding are practiced in horticultural crop improvement. The fruit crops are long-lived perennials and are mostly tall, woody plants, so some specific problems arise in their breeding. (Hanke & Flachowsky, 2012). So, the horticultural crops have a long juvenile phase, which is characterized, apart from their habitus and some morphological properties, by a greater readiness to form adventitious roots and an inability to form flowers, fruits, and seeds (Doorenbos, 1965; Brewer & Palmer, 2011). The length of the juvenile phase under conventional natural conditions for apple and pear fruits extends from at least 6 years to 12 or more years (Nocker & Gardiner, 2014). The juvenile phase prolongation (with easy rooting) is necessitated for vegetative propagation by stem cuttings (rooting a severed stem of the parent plant) and layering (covering stems or runners with soil to cause adventitious roots). In contrast, horticultural crop breeders, on the other hand, attempt to accelerate the onset of the adult phase to obtain seeds and seedlings (Doorenbos, 1965; Migicovsky et al., 2021).

To this must be added the time needed to grow a progeny and for estimation of large populations of fruit tree seedlings to maturity, perhaps another above five years. Other physiological problems which retard the progress of fruit improvement include self-incompatibility, polyploidy, and parthenocarpy (Mullins, 2006).

The adult trees develop flower buds with the stage of blossoms. The apple and pear flower structures are similar to those of most allogamous plants. Their flowers are hermaphroditic, with enough pollen and nectar for insects to land on them; hence, they are adapted to pollination by insects (entomophily). Flower pollination starts when the flowers open. Despite the apple and pear flowers are hermaphroditic their cross-pollination is ensured by the gametophytic self-incompatibility system. The gametophytic self-incompatibility is controlled by multiple alleles of the same gene, which in these plants have been situated at a single chromosomal locus and generally result in delayed pollen tube growth following self-pollination, but inhibition of pollen tube growth may show in the stigma or in the ovary (Sedgley, 1994).

Malus and *Pyrus* flowers are similar, but arrangements of florets and flowering sequences in their inflorescence are different. The *Malus* inflorescence (cyme) is basipetal (centrifugal), terminal flowers evolve more rapidly than lateral flowers; with flowers development, and opening from the apex towards the base; instead, *Pyrus* corymbs are acropetal (centripetal), with development, maturation, or opening of flowers from the side-lateral (base) toward the terminal flowers (Cronk, 2009; Gur, 1985).

The breeders in creating new cultivars of apple and pear as traditional source material for clonal selection until recently used chiefly existing genotypes. Clonal selection is the most simplistic approach to the development of improved cultivars of asexually propagated horticultural species (Acquaah, 2012). Therefore, their cultivars are selected mainly as the vegetative progeny of chance seedlings or as a result of the asexual propagation of spontaneous somatic mutations. For artificial clonal selection for new cultivars of apple and pear development, are also used the intraspecific, interspecific, and intergeneric hybrids as a source of starting materials. The artificial crosses of selected parents are one of the principal techniques of maximum genetic variability induction and production of superior recombinant genotypes that combine the desirable characteristics of the parents (Bertan et al., 2007; Orton, 2019a,c). The analysis of second-generation hybrid populations aims to a selection of seedlings with desirable traits, individuals which are characterized by the presence of identical recessive allele pairs, hitherto it is almost not used in apple-tree and pear-tree breeding (Opalko et al., 2005). The point is that because of the inbreeding depression and gametophytic self-incompatibility (Brown, 2003; Del Duca et al., 2019; Orton, 2019c; Sattar et al., 2021) in *M. domestica* and *P. communis* cultivars the fertilization and fruit set are restricted in the self-pollination condition. Some authors recommend the use in the capacity of second-generation hybrids of seedlings grown from seeds obtained from free pollination of flowers of first-generation hybrids. However, such progeny cannot be considered second-generation in the genetic sense, but only of the new first-generation obtained from pollination of the hybrid individual flowers by pollen of numerous unknown male parents (Opalko & Zaplichko, 1981).

The method has not yet become widespread due to the above difficulties (Brown, 2003; Mullins, 2006; Orton, 2019c), although the possibility of using controlled inbreeding to obtain a second-hybrid generation in perennial woody plants to create new source material already was reported in publications of the 1930-s. Ukrainian scientists returned to the practical use of controlled inbreeding (in an improved version) in the 1980-s. The essence of the improvement was the induction of genetic diversity at self-incompatibility alleles in the pollen-grain populations used for artificial self-pollination, by mutagenesis induced (Opalko & Zaplichko, 1981).

Since apple and pear cultivars are mostly clones, which are reproduced on rootstocks, each cultivar-clone, obtained by vegetative propagation of a hybrid seedling or mutant, consists of individuals identical in genotype and with almost equal homogeneous phenotype and economically valuable characteristics. Such identity can be disrupted by somatic mutations, a small proportion of the best of which can be deliberately propagated by breeders, and plants carrying mutant genes that control the expression of desired traits can become the progenitors of new cultivars-clones created by clonal selection. However, if a mutation arises in one from many cells within a multicellular organism, and the mutated and unmutated cells simultaneously propagate by mitosis, then they give to a chimera (a plant composed of tissue containing two or more genetically distinct cell types). From the chimeric tissue, one or more wholly mutated buds may develop into an independent, completely mutant stem (Lamo et al., 2017). In addition, the vast majority of somatic mutations are harmful or even baneful for plant vitality and adaptability and reduce the quality of horticultural crops, so their carriers are not used by nursery laborers for cultivars' vegetative propagation in the horticultural nursery.

The plants of apple and pear cultivars are heterozygous, and their main economically valuable characteristics are controlled polygenically. This makes it necessary to create a large amount of source material for selection since there are very few forms with the desired characteristics in the population of breeding seedlings. In particular, according to America's most famous horticultural breeder, Luther Burbank (1849–1926), at least 20,000 seedlings are needed to produce a new improved cultivar with the desired characteristics. He recommended rigorous rejection of worse breeding material in the early stages of breeding work, leaving one or two of the most promising seedlings. It can be said that Luther Burbank intuitively followed the modern rationale of plant breeding still more than a hundred years ago (Janick, 2015).

The results of the practical work of modern breeders show that the probability of obtaining a cultivar from the original populations in fruit and berry crops is low and does not exceed 0.3–0.5% of the total number of hybrid or mutant seedlings within the populations studied. Thus, a breeder needs much arable land to grow several thousand garden seedlings for testing offspring in a breeding nursery and breeding garden. The cultivation of such large areas and the care of breeding orchards require a lot of labor and energy resources. Therefore, the search for opportunities to significantly reduce the amount of work in the early stages of the breeding process, as well as reducing the generation cycles of improved plants, is among the most important tasks of breeding major horticultural crops (Opalko et al., 2005).

That is why, to reduce the amount of work at the stages of growing and testing hybrid seedlings and at the early stage of identification and selection of mutant clones, we have developed a novel breeding approach to the woody horticultural crops for the improvement of apple and pear cultivars. At the stage of creating the starting material for clonal selection in the proposed breeding scheme, individual elements of inbreeding in the form of geitonogamy (Snow et al., 1996) were combined with elements of mutational breeding. According to this scheme, a significant reduction in the volume of work in the early stages of the breeding process was to be achieved through the use of self-incompatibility mechanisms for the para-natural selection of mutant pollen grains at the pistil receptacle during their germination. The working hypothesis towards controlled geitonogamy experiments was the assumption that the penetrance of *S*-alleles, typical for *M. domestica* and *P. communis* cultivars, can be modified when used for inbreeding pollen from the same plant, but pre-treated with mutagens (Opalko & Zaplichko, 1981; Opalko et al., 2005). The *M. domestica* and *P. communis* flowers are hermaphroditic, and their cross-fertilization (allogamy) is provided by gametophytic self-incompatibility, in which any of the pollen grains with an *S*-allele present will not produce a pollen tube in the pistil, and consequently, they will not fecundate the ovule with an even *S*-allele present. Such self-incompatibility phenomena are determined by the pollen haploid (gametophytic) genome exposed only after the pollen grain's germination (Bisi et al., 2019; Del Duca et al., 2019; Fujii et al., 2016; Hoebee et al., 2012). A pollen tube with an S-allele (self or foreign) that matches one of the two S-alleles contained by the pistil is rejected (Franceschi et al., 2012). Provided that the pollen donor and the pollen recipient share both *S*-alleles at the self-incompatibility locus (e.g., S_1S_2 × S_1S_2), all pollen will be rejected and no fertilization occurs. On the contrary, if they have different *S*-genotypes, that is, share no *S*-allele (e.g., S_1S_2 × S_3S_4 or the pollen recipient with some allele S_n), the cross is fully compatible independently of dominance effects among *S*-alleles (Hoebee et al., 2012).

It is fairly obvious that any new *S*-alleles (e.g., S_n) can arise by mutation (Abe et al., 2023; Claessen et al., 2019) and by genome editing and cisgenesis (Domenichini et al., 2023). Therefore, we applied pollen mutagenic treatment to induce *S*-allele diversity in *M. domestica* and *P. communis* pollen grains. It is assumed that self-compatibility could be attained after novel mutations in *S*-alleles have arisen. However, the display of self-compatibility after pollen mutagenic treatment may be the result of both genetic and physiological effects. Genetic effects may be due to mutations in *S*-genes and/or some gene modifiers that inhibit the *S*-alleles of the self-incompatibility gene. Instead, physiological effects are obvious in the stimulation of pollen tube formation and their subsequent growth stimulated by the influence of mutagen-modified enzyme systems in the actual pollen grain (pseudo-self-compatibility). Although physiological changes are not inherited, they can promote fruit and seed set as well as improve the quality of seeds during inbred (geitonogamous) pollination. For the selection of pollen grains activated by mutagenic treatment, elements of natural selection (para-natural selection) can be used when only pollen-grains activated germinate on the stigma (Opalko & Zaplichko, 1981; Opalko et al., 2005). This article is devoted to the study of the possibilities of obtaining inbred generation of apple and pear cultivars by pollination with own (collected from the flowers of the same plant) pollen, pre-treated with gamma-rays and chemical mutagens in the "gaseous phase".

Materials and Methodology. The studies of the controlled geitonogamy were performed at the apple and pear orchards of the National Dendrological Park "Sofiyivka" of the NAS of Ukraine and the Uman National University of Horticulture. The experience orchards are located in the Uman'-Man'kivka area of the Central Pre-Dnipro Upland of the East European plain of the Podilsk-Prydniprovsk area of the Forest-Steppe Zone of Ukraine. This area is in central Ukraine, in the southwest of the Cherkasy region. It is in the basin of the South Bug River at the junction of two small rivers, Kamyanka and Umanka (Gerasymenko et al., 2007). This area is localized in the Atlantic continental climatic region, which determines its climate as temperate-continental, relatively warm, with a positive mean annual air temperature of +7.0°C to +7.7°C. The area is characterized by unstable humidification and considerable temperature fluctuations. January is the coldest month. The average January air temperature ranges from -5.6°C to -6.1°C. An absolute minimum of air temperature reaches -34°C (January 9, 1987). During winter, there are frequent thaws, which can often last for more than 5 days; in some years, the number of days with a thaw reaches 75. A frost-free period with a daily mean air temperature above +5°C continues on average for 205–210 days (Lipinsky et al., 2003).

According to long-term data from the Uman meteorological station, the average annual rainfall is 633 mm. During the year, both during long-term observations and in the years of research, more precipitation fell during its warm period. However, in some years the maximum and minimum amounts of precipitation may be observed in other months.

Our *Malus* collection consists of approximately 60 species and intraspecific taxa, including 40 taxonomic units of ornamental crabapples. The *Pyrus* collection covers more than 60 species and about ten intraspecific taxa, without seedlings of our breeding (Opalko et al., 2020).

The experiments were performed on fruiting 10–20-year-old apple trees ‘Golden Delicious’, ‘Zymove lymonne’, ‘Cortland’ and ‘Slava Peremozhcyam’, as well as pear trees ‘Bere Desiatova’ and ‘Clapp's Favorite’(=‘Uliublena Klappa‘). Castrated flowers of these cultivars were pollinated with pollen from other flowers of the same tree, that is, geitonogamy was performed (one of the variants of inbred pollination), and also a series of variants of geitonogamy by mature pollen preliminarily treated with gamma rays in doses of 5; 20, and 50 gray (Gy) and chemical mutagens in the "gaseous phase" (Kushi et al., 1983) during 72 hours of exposure was done. That treatment duration is long enough to permit infusion of the mutagen to pollen grains. Treatment of pollen with chemical mutagen vapors was performed in ten-liter sealed desiccators, in which open tubes with pollen and mutagens were placed: nitrosodimethylurea (NDMU) — 50 mg per desiccator; dimethyl sulfate (DMS) and ethylene oxide (EO) — both 12 drops per desiccator. The statistical analysis and the calculation of the least significant differences (LSD_{05}) between the experimental data were carried out by the methods of Ronald Aylmer Fisher (Fisher, 2006).

Results and Discussion. The mutagens’ efficiency was confirmed in most variants of the experiment with apple (*M. domestica*). In the control variants (open pollination), the studied apple cultivars had an average of 13.00–27.93% of fruits, in which an average of 6.48–15.44 seeds were formed. 3.60–7.30% of fruits were formed from geitonogamy by untreated mutagen pollen in apple trees. The result of fruit set in *M domestica* cultivars analysis showed the dependence of this indicator on the genotype. Although in all variants with mutagens, the fruit and seed set significantly exceeded variants of "geitonogamy without mutagen treatment" (table 1) the data received give not the possibility to determine unequivocally patterns of influence of the mutagen treatments and gamma-radiation dose for fruit and seed set. Instead, the rate of seed attachment depended on both genotype and gamma-ray dose and the form of chemical mutagens. For example, ‘Zymove lymonne’ failed to obtain seeds in the geitonogamy without mutagen treatment variant, while ‘Golden Delicious’, ‘Cortland’ and ‘Slava Peremozhcyam’ budded an average of 2.67 to 3.75

seeds per fruit. In mutagenic variants, 2.28–7.04 seeds per fruit were formed in all cultivars.

Table 1. Average apple (*Malus domestica*) cultivars fruit set and an average number of seeds per one fruit with geitonogamy by pollen mutagens treated

Treatment	Average fruit set, %				Average number of seeds per fruit			
	‘Zymove lymonne’	‘Slava Peremozhcyam’	‘Golden Delicious’	‘Cortland’	‘Zymove lymonne’	‘Slava Peremozhcyam’	‘Golden Delicious’	‘Cortland’
Open pollination (control)	13.00	18.44	27.93	12.48	6.48	15.44	10.30	10.15
Geitonogamy without mutagen treatment	3.60	2.74	7.30	1.55	0	2.67	2.99	3.75
Gamma rays, 5 Gy	5.00	4.00	14.71	8.14	2.39	3.17	5.43	6.19
20 Gy	3.90	5.46	14.10	8.07	3.00	4.17	6.39	7.02
50 Gy	3.70	4.66	13.29	5.97	3.81	4.95	6.70	6.10
NDMU	4.51	4.86	15.62	4.81	2.83	6.12	6.05	7.04
DMS	4.39	5.87	14.30	3.27	3.69	8.40	6.71	6.69
EO	4.10	5.33	12.40	3.29	2.28	5.80	5.52	6.16
LSD_{05}	0.14	0.29	1.08	0.11	0.12	0.27	0.31	0.38

Indicators of apple cultivar seed germination with geitonogamy by pollen mutagens treated depended on genotype, dose, and type of mutagen (table 2). In the experimental variants with geitonogamy without mutagen treatment, the average percentage of seed germination of ‘Cortland’ and ‘Slava Peremozhcyam’ was about 60, but ‘Golden Delicious’ had only about 30. The best irradiation variant for ‘Zymove lymonne’ and ‘Cortland’ was a dose of 5, for ‘Slava Peremozhcyam’ — 20, and for ‘Golden Delicious’ — 50 Gy. Among the chemical mutagens, nitrosodimethylurea and dimethyl sulfate were preferred, while ethylene oxide reduced the germination of geitonogamous seeds of all cultivars.

Seedling survival depended more on genotype than on mutagen. The highest percentage of viable seedlings came from ‘Golden Delicious’, slightly less from ‘Slava Peremozhcyam’ and ‘Zymove lymonne’, while from ‘Cortland’ twice fewer seedlings survived than from ‘Golden Delicious’.

In all variants with mutagens, various morphological changes were observed both directly in the year of treatment and in the following years. Along with normal seeds, there were seeds one and a half–times or two times smaller, although well-filled and quite germinable, as well as large seeds of modified shape (spherical, ribbed, hilly) and paired seeds — "twins". The frequency of such variations increased in variants with gamma-rays (doses of 20 and 50 Gy) and chemical mutagens.

Table 2. Average seed germination and seedling survival percentage in apple (*Malus domestica*) cultivars with geitonogamy and pollen mutagens treatment

Treatment	Seed germination, %				Seedling survival, %			
	‘Zymove lymonne’	‘Slava Peremozhcyam	‘Golden Delicious’	‘Cortland’	‘Zymove lymonne’	‘Slava Peremozhcyam	‘Golden Delicious’	‘Cortland’
Open pollination (control)	69.03	79.14	43.46	63.97	48.67	56.52	53.09	33.33
Geitonogamy without mutagen treatment	—	60.35	29.76	58.01	—	38.29	40.00	30.19
Gamma rays, 5 Gy	38.80	36.25	24.62	68.05	39.12	36.60	53.96	29.67
20 Gy	23.97	39.22	20.62	36.66	21.65	31.80	33.33	18.21
50 Gy	23.30	28.54	31.49	50.43	18.69	27.20	39.74	14.65
NDMU	52.81	21.79	59.14	49.58	36.66	41.61	51.03	21.66
DMS	50.00	29.77	50.43	34.40	30.03	40.14	49.66	16.33
EO	16.70	29.91	19.89	17.50	28.21	34.65	22.03	18.09
LSD_{05}	5.33	3.44	3.17	3.91	6.96	3.14	3.66	2.03

From geitonogamous seedlings ‘Cortland’ and ‘Slava Peremozhcyam’ in dimethylsulfate variants, individual tricotyledonous seedlings (with tree cotyledons) were obtained each year, from which normal plants were subsequently formed, the same as from dicotyledonous seedlings. Such changes have obviously the nature of morphoses, phenocopies, and other non-hereditary modifications. Chlorophyll mutations occurred in all variants. In some cases, plants with chlorophyll defects were stored until the age of one year. In seedlings of cultivars and forms with known pedigrees, which grew from seeds obtained from geitonogamy with both mutagen-treated pollen and from geitonogamy untreated mutagen pollen, signs of ancestral forms were observed in new combinations.

Most of these forms, including those with economically valuable characteristics, appeared in ‘Slava Peremozhcyam’ and ‘Golden Delicious’. So ‘Slava Peremozhcyam’ which is characterized by light green fruits with a solid, elegant red, blurred blush, is a cultivar selected from progeny of crossing between the popular early summer season apple cultivar ‘Papirovka’ (female parent) and the late-autumnal cultivar, the national apple of Canada, ‘McIntosh’ (male parent). In the geitonogamy-mutant (NDMU treated) generation ‘Slava Peremozhcyam’ a seedling with snow-white fruits as in ‘Papirovka’ was selected, but the fruit shape of this heterogamous-mutant seedling was similar to the fruits of ‘Slava Peremozhcyam’ and had a wonderful aroma from ‘McIntosh’.

Experiments with pear (*P. communis*) also confirmed the effectiveness of the mutagens used in most variants of the experiment (table 3). In the variant of geitonogamy without mutagen treatment, in ‘Bere Desiatova’ an average of 0.84% of fruits were set, but all of them were seedless. In the variant of gamma rays at a dose of 5 Gy, the number of fruits was almost half compared to geitonogamy without the gamma irradiation variant, and as in the previous variant, all fruits were seedless. Within ‘Clapp's Favorite’, on the other hand, in the geitonogamy without mutagen treatment variant, almost as many fruits as in ‘Bere Desiatova’ appeared, but they had an average of 2.02 seeds, which is a little, but enough to produce descendants. Irradiation of ‘Clapp's Favorite’ pollen with gamma rays at a dose of 5 Gy ensured seed germination of 5.70%, which significantly exceeded that of the open pollination control variant.

Table 3. Average pear (*Pyrus communis* L.) cultivars fruit set and an average number of seeds per one fruit with geitonogamy by pollen mutagens treated

Treatment	Average fruit set, %		Average number of seeds per fruit	
	‘Bere Desiatova’	‘Clapp's Favorite’	‘Bere Desiatova’	‘Clapp's Favorite’
Open pollination (control)	5.94	4.09	3.20	4.11
Geitonogamy without mutagen treatment	0.84	0.75	0	2.02
Gamma rays, 5 Gy	0.48	5.70	0	5.50
20 Gy	3.43	0.97	8.16	8.50
50 Gy	4.36	1.25	9.33	8.06
NDMU	0.83	1.47	5.00	7.85
DMS	1.43	1.22	5.31	7.97
EO	1.24	1.66	4.46	5.12
LSD_{05}	0.21	0.17	0.23	0.24

Further study of mutant-inbred generations of pear cultivars made it possible to select several economically valuable mutations, among which were seedlings with limited growth (dwarf and semi-dwarf trees), altered fruit color, taste, and different terms of maturity.

The comparison of apple (*M. domestica*) and pear (*P communis*) cultivars studied on the indicators of fruit and seed germs (see tables 1 and 3) showed that the average fruit set and an average number of seeds per one fruit of apple cultivars was much higher than in pear cultivars. Usually, even during open pollination, most of the studied apple cultivars budded about 10 seeds per fruit, although in the fruits of some cultivars, such as ‘Slava Peremozhcyam’, 12 to 20 seeds or more were formed annually, and in ‘Zymove lymonne’ three times less (4–7 seeds per one fruit). Instead, the studied pear cultivars in the control variants (with free pollination) tied an average of 4.09–5.94% of fruits, which develop an average of 3.20–4.11 seeds, in other words, the indicators of pear cultivars were 4–5 times lower (by fruit) and 3–4 times lower (by seeds).

Such an advantage of apple was observed in open pollination variants; geitonogamy without mutagen treatment; and in most variants of geitonogamy by pollen mutagen treated. This difference can be explained by diversities in the structure of apple and pear inflorescence, as well as the sequence of anthesis. So, the apple inflorescence is a cyme of 4–6 flowers, and where individual flowers bloom begins with a terminal flower, then the basal lateral flower and the rest of the flowers bloom (Pratt, 1988). This is a basipetal type of inflorescence development. Instead, according to our observations, pear cultivars are more characterized by an acropetal order of flowering, i.e., the lower flower is the first to open in the inflorescence. Large basal flowers more often set the fruit than smaller ones located higher in the cyme. Terminal flowers also develop few fruits, although they can be pretty large.

The capacity for the apple and pear flowers to set fruit and to seeds per fruit can be explained by the physiological balance between flowers within the inflorescence (Lauri & Térouanne, 1999). The percentage of the average fruit set was calculated as the ratio of the number of ripe fruits to the number of flowers in the inflorescence. Therefore, in multi-flowered inflorescence with the same number of ripe fruits, the percentage of the average fruit set will always be lower, as well as the number of seeds. In addition, the number of ripe fruits in the inflorescence, as well as on the tree, very much depends on the growing conditions not only in the harvesting year but also on the conditions of the previous year, when the laying and differentiation of flower buds. The number of ripe fruits in the inflorescence, as well as on the tree, is also determined by the characteristics of individual cultivars.

Conclusions. The fact of an increasing percentage of seed set, resulting from geitonogamy pollen, pre-treated with gamma-rays and chemical mutagens, can be explained from physiological-biochemical and genetic-mutational positions and, accordingly, explain the emergence of generation with new characteristics (relative to the original mother plant). In the case of stimulation, such neoplasms can be mainly considered to be a result of cleavage of a heterozygous maternal individual, whereas, among the neoplasms in variants treated with mutagens, both mutations and cleavage results may occur. In fact, the inbreeding itself can also be an inducer of the mutation process, especially in self-incompatible self-fertilization, and affect the frequency and spectrum of neoplasms. Therefore, geitonogamy with pollen pre-treated with mutagens can be used to produce an inbred generation of such self-incompatible species as *M. domestica* Borkh. and to induce genetic variability. Finally, the technique of obtaining an inbred generation of apple and pear cultivars by pollination with their pollen, pre-treated with gamma-rays and chemical mutagens in the "gaseous phase" can be recommended for apple (*M. domestica* Borkh.) and pear (*P. communis* L.) breeding, and also deserves to be studied on other cultivated plants with self-incompatibility genes.

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