Cytological characteristics of birch and alder pollen (different breeding forms) used in hybridization

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Abstract. Hybrids of some species of birch trees form completely sterile seeds (laboratory germination is zero in some hybrids, such as Karelian birch \times Chinese white birch of inbred and outbred origin). It was noted analyzing the size distribution of birch pollen. Six size classes of pollen grains were identified, the modal values of which are arranged in increasing order as follows: 17 µm, 22 µm, 28 µm, 33 µm, 39 µm and 44 µm. The process of development of the male generative sphere proceeds without significant deviations in the studied black and gray alder trees. It ends with the formation of high-quality, aligned pollen. The obtained data indicate that one group of trees show normal formation of the reproductive sphere. It indicates the reasonableness of including them in the breeding process.

1. Introduction

Plant pollen (or male gametophyte) is an important object of study from the point of view of plant systematic and molecular biology (since it has a haploid set of chromosomes).

Currently there are the following types of plant reproduction: (i) Apomixis. It is plant asexual reproduction through seed. Progeny of an apomictic plant are genetically identical to the maternal plant. (ii) Embryo sac. It is multicellular female gamete-producing structure of a flowering plant. It is also known as the female gametophyte, which is found in approximately 70\% of flowering plants. The diploid megaspore mother cell undergoes meiosis, forming four haploid megaspores. (iii) Apomeiosis. It is avoidance or failure of meiosis during the development of an embryo sac. (iv) Diplospory. (v) Apospory. (vi) Parthenogenesis [1].

The qualitative and quantitative composition of the forming pollen is largely determined by the dynamics and behavior of chromosomes in meiosis during microsporogenesis. The relationship between chromatin structure, chromosomes, and DNA damage and repair is discussed in the review [2]. It is also known that there is a direct relationship between the pollen grain sizes and their ploidy. Based on this, according to such a morphometric index as the diameter of pollen, one can indirectly judge the developmental characteristics of the male gametophyte of the studied organisms, their genetic nature, and the possible hybrid, mutagenic or polyploid nature. This is important when using trees as pollinators for hybridization.

The study of birch and alder pollen enables to simulate a forecast for its distribution and to determine the environmental assessment of urbanized areas. Pollen is also an object for studying the allergenic properties [3].
Doctors consider it to be the cause of allergic diseases - pollinosis, but despite this, we can not underestimate its useful properties. Pollen grains have a very rich composition compared to the other cells of the plant body. Birch pollen contains vitamins A, B₁, B₆, E, F, K, ascorbic and folic acids, trace elements, as well as phytoncides, which have a beneficial effect on the human body, ensuring its proper functioning. A certain qualitative and quantitative content of organic and inorganic compounds shows that pollen is a complex and diverse biochemical system.

Birch pollen has antioxidant, anti-inflammatory, hypolipidemic (anti-sclerotic), anti-prostatic, anthepatotoxic, and also antidote (after intoxication strong toxins) and anti-anemic effect. In recent years, other properties of pollen have been discovered and studied (mainly in animal tests): immunoregulatory, immunosuppressive, antiallergenic, antitumor, antiangiogenic (inhibits the growth of new blood vessels). It can be a painkiller when acting on the nervous system and urinary tract, and slowing the aging of human bodies [4]. Birch pollen is an excellent antibiotic, able to stop the development of many microorganisms that are difficult to destroy and are causative agents of many intestinal diseases [5].

2. Experimental part
The selection of a woody plant breeding system depends on their reproduction system. Birch and alder show both vegetative and seed propagation.

Functionally, pollen grain is a biological container that contains the male gametophyte. Such a container protects male gametes from the adverse effects of the environment during their transfer from anthers to pistil. Pollen grains of various plant species vary in size (from 10 to almost 100 micrometers) and in shape: round, oval, discoid, bean-shaped, and filamentous. The natural color of pollen is mostly white, cream, yellow or orange. According to the texture of the cell wall, pollen is also different. Its surface can be very smooth or it can be covered with different outgrowths (figure 1).

A number of studies on plant objects have shown that genomic mutants have different pollen sizes [6-8], and more stable results show measurements of Karelian birch pollen, which indirectly indicates its polyploidy and best adaptability to natural conditions. Measurements of Karelian birch pollen show the smallest stability, which indicates an unstable systematic position of this species [9]. Therefore, the determination of the size of pollen grains can be the first step in the identification of genomic mutants (haploids, polyploids, and mixoploids).

The objectives of this study were:
1. To study the influence of F₁ and I₁ pollination method in European birch and some hybrids with its participation on the heterogeneity (of different quality) of pollen grain sizes.
2. To identify the dependence of the pollen size on the phenotypic manifestation of the “karelian” sign in contrasting forms of Karelian birch with this trait.

3. Materials and methods

The following species and forms of woody plants were used in the experiment: Karelian birch (*Betula pendula* Roth var. carelica (Mercklin) Hämet-Ahti), Chinese white birch (*Betula albosinensis* Burkill), gray alder (*Alnus incana* (L.) Moench), black alder (*Alnus glutinosa* (L.) Gaertn), Japanese alder (*Alnus japonica* (Thunb.) Steud).

Mature pollen from three hybrids was studied: 1. Karelian birch 1 × Chinese white birch; 2. Karelian birch 2 × Chinese white birch; 3. Karelian Birch 4 × Chinese white birch.

- Seed progeny of two mother trees of Karelian birch (the first generation of outbred and inbred origin).
- Seed offspring of Karelian birch of different life forms. Table 6 presents data on pollen from Karelian birch trees without external manifestation of the trait.

Alder pollen harvesting: branches 0.5 - 1.0 m long with flower earrings to a time close to flowering in nature are cut. In this case, better pollen can be got. Cut branches are put in jars with water. Pollen “distillation” is carried out in a greenhouse or room. Humidity should not be below 80%. Air temperature is not higher than 18 °C. Dry air and high indoor temperatures dramatically reduce pollen viability [10–12]. It is advisable to renew the cuts twice a week and change the water. Male earrings are collected in inflorescences - densely flowered earrings. Earrings are drawn, and the pollen is poured out. It is collected in Petri dishes. Picking off earrings and drying pollen is not recommended. Pollen is stored in a dry, cool, dark place. Before hybridization, the viability of pollen was determined in the laboratory [13]: germination of pollen on artificial liquid media, solid media, and rapid methods (the reaction of dyes on vital enzymes and other substances). We used the fast method for determining the viability of pollen – pollen coloring with iodine-chloral hydrate. The reagent was prepared in 2-3 days: chloral hydrate (5 g) is mixed with 2 ml of water; crystalline iodine (0.2 g) is added. The mixture is left to infuse. This method is based on the iodine reaction: viable pollen grains are usually completely filled with starch. If the pollen body is colored completely with a reagent in the form of purple grains, then the pollen is considered to be viable. Half of the partially colored grains are classified as viable, the rest - as unviable ones. Uncolored grains are classified as unviable ones (figure 2).

![Figure 2. Pollen of black alder (a) and gray alder (b).](image-url)
4. Results and discussion
The express method gives an approximate percentage of pollen viability, and yet you can plan work on hybridization. Pollen is stored in bottles at the bottom of the refrigerator. The viability of alder pollen decreased 10–30% in seven months (table 1).

Table 1. The viability of black alder and gray alder pollen used for hybridization.

| Number of alder tree | The number of colored pollen grains (%) |
|----------------------|----------------------------------------|
|                      | April 15, 2018 | November 25, 2018 |
| Black alder 1        | 100           | 69               |
| Black alder 2        | 68            | 59               |
| Black alder 3        | 97            | 61               |
| Gray alder 1         | 91            | 72               |
| Gray alder 2         | 90            | 68               |
| Gray alder 3         | 95            | 74               |

In determining the viability of pollen by the express method, it was obtained that it was from 68% to 100% in freshly collected pollen.

Morphological indicators of the qualitative composition of alder pollen are presented in table 2.

Table 2. The diameter of pollen from the studied alder trees, black and gray.

| Number of alder tree | The diameter (µm) of pollen, the amount of pollen grains, % |
|----------------------|----------------------------------------------------------|
|                      | 23.8 – 26.7 | 29.7 – 32.7 | 35.6 | Deformed pollen |
| Black alder 1        | 2           | 90          | 6    | 2               |
| Black alder 2        | 16          | 78          | -    | 6               |
| Black alder 3        | 6           | 85          | 7    | 2               |
| Gray alder 1         | 48          | 45          | 4    | 3               |
| Gray alder 2         | 52          | 48          | -    | -               |
| Gray alder 3         | 30          | 60          | 2    | 8               |
| Japanese alder       | 81          | 19          | -    | -               |

The analysis of the final stage of microspore formation showed that in the six studied black and gray alder trees, the development process of the male generative sphere proceeds without significant deviations and ends with the formation of high-quality, aligned pollen. In black alder No. 1 and black alder No. 3, abnormal pollen grains, probably hyperaneuploids, with an increased number of microspore chromosomes, were identified along with normal ones. Small pollen grains (probably hypoaneuploid) are found.

The obtained data indicate that formation of the reproductive sphere proceeds normally in one group of trees. It indicates the advisability of including them in the breeding process. We can expect the appearance of different-quality offspring when using trees, where abnormal pollen grains have been identified.

When analyzing the size distribution of birch pollen, it was noted that the hybrids of some species of birch trees form completely sterile seeds (laboratory germination is zero in some hybrids, such as Karelian birch × Chinese white birch of inbred and outbred origin). Six size classes of pollen grains

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were identified, the modal values of which are arranged in increasing order as follows: 17 µm, 22 µm, 28 µm, 33 µm, 39 µm and 44 µm (table 3).

Hybrid of Karelian birch × Chinese white birch (table 3) has pollen of three size classes: 22, 28 and 33 µm. In three hybrids, Karelian birch 2 × Chinese white birch the following pattern was seen: all pollen grains were divided into two groups according to the morphological parameters. The first group includes the pollen grains which are mainly 22 and 28 µm in size. No heterogeneity was detected in the second group with a smaller predominance of smaller pollen.

**Table 3.** The ratio of the main size classes of pollen grains in hybrids on the basis of European birch.

| Variant of crossing                        | Frequency of occurrence, % |
|-------------------------------------------|----------------------------|
|                                            | 17 | 22 | 28 | 33 | 39 | 44 |
| Karelian birch 1 × Chinese white birch    | 6  | 64 | 30 |    |    |    |
| Karelian birch 1 × Chinese white birch    | -  | 2  | 64 | 34 | -  | -  |
| Karelian birch 2 × Chinese white birch    | -  | 56 | 44 | -  | -  | -  |
| Karelian birch 2 × Chinese white birch    | -  | 52 | 46 | 2  | -  | -  |
| Karelian birch 2 × Chinese white birch    | -  | 5  | 6  | -  | -  | -  |
| Karelian birch 4 × Chinese white birch    | -  | -  | 14 | 56 | 22 | 8  |

Karelian birch 4 × Chinese white birch hybrid showed significant pollen size polymorphism. The modal class had pollen sizes of 33 µm. An examination revealed large single pollen grains with a diameter of 44 µm. The distribution of pollen grain size classes is close to normal one.

According to the results of a comparative study of the laboratory germination of inbred and outbred seeds, the mother tree of Karelian birch 11 was classified as self-sterile (germination of inbred seeds is zero). This tree was characterized by medium-sized pollen. However, pollen was heterogeneous in composition in one tree (table 4).

Tree number 18 is self-sterile and has smaller pollen.

**Table 4.** The ratio of the main size classes of pollen grains in the seed offspring of Karelian birch produced by different pollination methods.

| Origin                          | Frequency of occurrence, % |
|---------------------------------|----------------------------|
|                                 | 17 | 22 | 28 | 33 | 39 | 44 |
| Karelian birch 11 self-pollination | -  | 22 | 72 | 4  | 2  | -  |
| Karelian birch 11 self-pollination | -  | 48 | 48 | 4  | -  | -  |
| Karelian birch 11 self-pollination | -  | 30 | 62 | 8  | -  | -  |
| Karelian birch 11 open pollination | -  | 30 | 70 | -  | -  | -  |
| Karelian birch 11 open pollination | -  | 74 | 26 | -  | -  | -  |
| Karelian birch 18 self-pollination | 4  | 64 | 32 | -  | -  | -  |
| Karelian birch 18 open pollination | 6  | 38 | 48 | 8  | -  | -  |
| Karelian birch 18 open pollination | -  | -  | 36 | 64 | -  | -  |
| Karelian birch 18 open pollination | -  | 16 | 62 | 22 | -  | -  |
| Karelian birch 18 open pollination | 4  | 64 | 32 | -  | -  | -  |
In the progeny of Karelian birch of different life forms (tables 5, 6) there is a shift of the pollen morphometric parameters towards smaller classes (the most numerous was a class of 22 µm). Pollen heterogeneity was also small.

**Table 5.** Ratio of the main size classes of pollen grains in Karelian birch of different life forms.

| Life form            | Frequency of occurrence,% |
|----------------------|---------------------------|
|                      | 17 | 22 | 28 | 33 | 39 | 44 |
| Standard 1           | -  | 64 | 36 | -  | -  | -  |
| Standard 2           | -  | 70 | 30 | -  | -  | -  |
| Standard 3           | -  | 84 | 16 | -  | -  | -  |
| Standard 4           | -  | 86 | 14 | -  | -  | -  |
| Lyre-shaped 1        | -  | 50 | 50 | -  | -  | -  |
| Lyre-shaped 2        | -  | 90 | 10 | -  | -  | -  |
| Semi-bush            | -  | 24 | 64 | 12 | -  | -  |
| Bush                 | -  | 66 | 34 | -  | -  | -  |

**Table 6.** Control trees of Karelian birch (without external expression of the trait).

| Tree    | Frequency of occurrence,% |
|---------|---------------------------|
|         | 30 | 56 | 8  | 3  | 3  |
| Tree 1  | -  | -  | -  | -  | -  |
| Tree 2  | -  | 56 | 44 | -  | -  |
| Tree 3  | -  | 50 | 50 | -  | -  |

5. Conclusion

The obtained data may indicate that an increase in pollen size does not affect the viability of inbred birch seeds. Moreover, self-sterile form of Karelian birch No. 18, which was characterized by small pollen grains, produced a small amount of full-fledged inbred seeds, and later seedlings and trees. The characteristic of black and gray alder pollen showed that the development of the male generative sphere proceeds without significant deviations and ends with the formation of high-quality, leveled pollen suitable for the hybrid production.

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