Comparative characteristics and germination of *Pinus sibirica* seeds collected from places of natural growth and in the St. Petersburg Botanical garden of Peter the Great

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**Abstract.** In this article sowing qualities of Pinus Sibirica seeds are studied as well as the effectiveness of various germination stimulants. The seeds were collected in various regions of growth, such as Novosibirsk, Republic of Tyva and St. Petersburg’s Botanical garden of Peter the Great. Also, strobil biometrics and digital x-ray analysis of seeds are presented. The results of application of 6 different stimulators of seed germination according to their absolute and soil germination, germination dynamics, growth biometric indicators are presented as well.

1. **Introduction**

Siberian pine (*Pinus sibirica* Du Tour.) has its own biological features: slow growth at a young age, high variability in crown diameter and its height, high frost resistance, and it is not demanding on soils, etc. *Pinus sibirica* may be recommended in landscape gardening culture for its decorative effect [1]. It is well-known fact that of all coniferous forests, cedar forests are the most difficult to restore naturally [2]. The first artificial breeding of *Pinus sibirica* in the European part of Russia dates back to the second half of the XVI century, when the Tolgskaya cedar grove was founded 8 km from Yaroslavl. Apparently, it is the world's first culture of *Pinus sibirica* outside its range [3]. *Pinus sibirica* has been in the culture of St. Petersburg since the city was founded [4]. Despite many years of experience in cultivating *Pinus sibirica* in St. Petersburg and Leningrad region, it still remains quite a rare highly decorative plant with edible seeds. The main way of *Pinus sibirica*’s reproduction is propagation by seeds. Known methods of seed propagation offer autumn sowing of seeds or stratification for 3-6 months [5], pre-sowing treatment with growth regulators followed by stratification or without it [6, 7]. Therefore, the quality of seeds and ease of growing planting material from them is an important factor in the distribution of *Pinus sibirica* in the North-West of Russia.
2. Methods and Materials

Objects of a research were *Pinus sibirica* seeds collected in places of natural growth: vicinities of Novosibirsk (sample 1), the Republic of Tyva (sample 2) and collected in the St. Petersburg Botanical garden of Peter the Great on site 127 (sample 3).

To implement the method of microfocus seed survey specialists from Agrophysical research Institute and Saint Petersburg Electrotechnical University "LETI" developed hardware-software complex on the basis of a traveling x-ray diagnostic installation PRB-02 for quality control of seeds. The developer and manufacturer is "ELTECH-Med", Saint-Petersburg, Russia [8].

The magnification factor of the image was 3.0 x for x-ray.

Software processing of digital images of seeds was carried out in the program ARGUS-BIO. The following parameters were analyzed: % of full-sized seeds, the embryo Area (mm^2), the area Ratio of the fetus to thalalus(%), a Factor of the ellipse of the embryo (Rel. units), the Area of the endosperm (mm^2), the relative area of the detachment of the shell (%), the relative area of the shell (%), the relative area of the endosperm (nucleus)(%), the relative area of the embryo(%), the Ratio of the average brightness of the embryo to the average brightness of the projection of the seed (Rel. units) [9].

Various growth-regulating substances were used for seed germination: 3-indolylbutyric acid (IBA) and α-naphthaleneacetic acid (α-NAA) (Sigma-Aldrich), sodium humate (FASCO), original multi-component preparations AB-7 and N-8B developed in the framework of the study of adaptation processes of deciduous and coniferous trees in the Arctic and subarctic climatic zones on the instructions of the Department of science and innovation of YANAO (state contract No. 01-15/4 of 25 July 2012). Their development and testing were carried out in 2012-2017. The drug AB-7 is designed as an adaptogen for transplanting plants from more southern regions in the Arctic. The basis for the development of China has become the drug of ABT-7 (Auxin Bequeathed with a Third component) [10] developed in the Chinese Academy of Forestry in 1992, the drug AB-7 is a solution of potassium salts of some amino acids, vitamins and plant growth regulators. The drug N-8B in its composition has an original product 3-(6-amino-3H-purine-3-il)-propane-1,2-diol having cytokinin activity. Cytokinins participate in many physiological processes of plants, regulate cell division, morphogenesis of shoot and root, chloroplast maturation, linear cell growth [11]. It is synthesized by the method [12]. Also in its composition N-8B contains a number of vitamins, amino acids and other BAS(biologically active substances). Due to the filing of an application for a patent, the formulas of both drugs are not disclosed in detail in the article.

To study the seed reproduction of *Pinus sibirica*, we conducted a number of experiments with seeds from the places of natural growth and from the St. Petersburg Botanical garden of Peter the Great. For germination of seeds we used the following conditions. One experiment took 100 seeds. Seeds were kept in solutions for 72 hours at 18-20°C, then immediately sown in open ground. For seed germination, a mixture of garden soil, deoxidized peat and sand in a ratio of 1:1:1 was used.

Calculation of statistical characteristics of samples in the study of qualitative characteristics was carried out according to B A Dospekhov [13].

3. Results and Discussion

Table 1 presents biometric indicators of cones and seeds of *Pinus sibirica* from places of natural growth, and from the St. Petersburg’s Botanical garden of Peter the Great.

Samples of cones from the vicinity of Novosibirsk were characterized by the best biometric indicators in comparison with samples from the Republic of Tyva and St. Petersburg’s Botanical garden of Peter the Great, namely, the maximum average weight of cones 45.26 ± 2.075 mm, average width of cones 65.07 ± 3.178 mm, and the average weight of seeds in the cone of 25.76 ± 1.714 g.

Sample from St. Petersburg, Botanical garden had the worst biometric indicators, such as average length of cones 75.18 ± 3.362 mm, and number of seeds per cone 74.09 ± 3.807 units, in comparison with samples of cones from Novosibirsk and the Republic of Tuva.
Table 1. Biometrics of *Pinus sibirica* seeds and cones.

| Parameter                        | Sample 1            | Sample 2            | Sample 3            |
|----------------------------------|---------------------|---------------------|---------------------|
| Places of growth                 | Novosibirsk         | Republic of Tyva    | St. Petersburg, Botanical garden |
| Date of gathering                | September 2016      | September 2016      | September 2017      |
| Average weight of 1 cone, g      | 45.26 ± 2.075       | 39.61 ± 0.880       | 39.63 ± 0.990       |
| Average length of 1 cone, mm     | 91.17 ± 5.005       | 83.67 ± 3.640       | 75.18 ± 3.362       |
| Average width of 1 cone, mm      | 65.07 ± 3.178       | 54.43 ± 1.274       | 51.82 ± 2.316       |
| Average quantity of seeds in 1 cone, units | 93.33 ± 2.040   | 90.37 ± 2.154       | 74.09 ± 3.807       |
| Average weight of seeds in 1 cone, g | 25.76 ± 1.714   | 20.73 ± 0.947       | 19.71 ± 0.991       |
| Average weight of seed scales, g | 19.49 ± 0.910       | 18.88 ± 0.600       | 19.94 ± 0.480       |
| Weight of 1000 seeds, g          | 276.98              | 230.33              | 266.35              |

To determine the biometric parameters of *Pinus sibirica* seeds, we used x-ray analysis. This method is used to determine the quality of coniferous seeds [14], in particular *Pinus sibirica* [15]. Digital X-ray images of *Pinus sibirica* seeds are presented at Figure 1.

![Figure 1](image-url)

Figure 1. Digital x-ray images of Siberian pine (*Pinus sibirica*): a – surroundings of Novosibirsk, b – Republic of Tyva, c – St. Petersburg Botanical garden of Peter the Great at lot 127.

The results of radiography analysis of *Pinus sibirica* seeds are presented in Table 2.

Table 2. Results of radiography analysis of *Pinus sibirica* seeds.

| Parameter               | Sample 1            | Sample 2            | Sample 3            |
|-------------------------|---------------------|---------------------|---------------------|
| Places of growth        | Novosibirsk         | Republic of Tyva    | St. Petersburg, Botanical garden |
| % of full-granular seeds| 93                  | 88                  | 78                  |
| Area of embryo, mm²     | 10.56 ± 0.422       | 6.97 ± 0.730        | 5.73 ± 0.473        |
soil seed germination increased, depending on the origin of the samples, in the range from 12.6 to 14.0% 3-indolylbutyric acid (IBA) to 56.8-71.0% (N-8B), compared with the control. Soil seed germination increased, depending on the origin of the samples, in the same sample, the maximum thickness of the seed shell is fixed.

The results of seed germinating of Pinus sibirica are showed in the Table 3.

| Plant growth-regulating chemicals | Concentration g/L (%) | Germination, % | Sample 1 Novosibirsk | Sample 2 Republic of Tyva | Sample 3 St. Petersburg Botanical garden |
|----------------------------------|-----------------------|---------------|----------------------|------------------------|----------------------------------------|
|                                  |                       | A  | B  | A  | B  | A  | B  | A  | B  | A  | B  |
| Control (water)                  | -                     | 2.1±2.81 | 2.0± | 0.5± | 0.5± | 1.3± | 1.0± |      |      |      |      |
| sodium                           | 1 (0.1)               | 49.5±9.80 | 46.0± | 35.2± | 31.0± | 46.0± | 37.0± |      |      |      |      |
| N-8B                             | 0.1(0.01)             | 73.1±8.69 | 68.0± | 56.8± | 50.0± | 69.2± | 54.0± |      |      |      |      |
| AB-7                             | 1.8(0.18)             | 64.5±9.38 | 60.0± | 51.1± | 45.0± | 61.5± | 48.0± |      |      |      |      |
| α-naphthylacetic acid (α-NAA)    | 0.12(0.012)           | 18.3±7.58 | 17.0± | 18.2± | 16.0± | 17.9± | 14.0± |      |      |      |      |
| 3-indolylbutyric acid (IBA)      | 0.1(0.01)             | 16.1±7.20 | 15.0± | 13.6± | 12.0± | 14.1± | 11.0± |      |      |      |      |

* Distinctions are significant at t=0.05 in comparison with control group

Experimental data showed high efficiency of application of various plant growth regulators on sowing qualities of Siberian pine seeds. Absolute seed germination increased, depending on the origin of the samples, in the range from 12.6 to 14.0% 3-indolylbutyric acid (IBA) to 56.8-71.0% (N-8B), compared with the control. Soil seed germination increased, depending on the origin of the samples, in
the range from 10.0 to 13.0% 3-indolyl butyric acid (IBA) to 53.0-66.0% (N-8B), compared with the control group.

Averaged data on seed germination of three samples of *Pinus sibirica* are presented in figure 1. Regardless of the seed batches, the best drugs were N-8B, AB-7 and sodium humate, since their use gave maximum germination. The use of standard auxin (α-NAA, and IBA) gave the lowest germination rate in all cases. Part of the seeds, not risen during the experiments, rose in the second year. In control experiments, the seeds have not actually risen. In these cases, we also observed seed germination in the second year.

The dynamics of germination of *Pinus sibirica* seeds was studied. In experiments using sodium humate, N-8B and AB-7 germination of seeds was completed within 30 – 50 days from the date of sowing. In other experiments, the gradual emergence of seedlings lasted for 70 days.

The data of dynamics of germination of *Pinus sibirica* seeds are presented in the Figures 3-5.

**Figure 2.** Influence of plant growth regulators on seed germination of *Pinus sibirica*.

**Figure 3.** Dynamics of seed germination *Pinus sibirica*, sample 1 – vicinities of Novosibirsk.
Figure 4. Dynamics of seed germination Pinus sibirica, sample 2 – Rep. of Tuva.

Figure 5. Dynamics of seed germination Pinus sibirica, sample 3 – St. Petersburg Botanical garden of Peter the Great at lot 127.

The seedlings of Pinus sibirica obtained as a result of experiments wintered the winters of 2017-2018 and 2018-2019 without losses and do not yield to the seedlings grown by literary methods in terms of biometric parameters. (Table 4).

Table 4. The size of seedlings Pinus sibirica in a year. Summary of experiences.

| Parameters                     | Sample 1 Novosibirsk | Sample 2 Republic of Tyva | Sample 3 St. Petersburg, Botanical garden | Sample 4 Scientific Experimental Station Otradnoye a |
|--------------------------------|-----------------------|----------------------------|-------------------------------------------|-----------------------------------------------------|
| Quantity of seedlings, units   | 208                   | 154                        | 128                                       | 57                                                  |
| Average height in 1 year, mm   | 51.6                  | 45.2                       | 48.7                                      | 45.5                                                |
| Maximum increment of 1 year of mm | 57.8                  | 51.6                       | 55.5                                      | 52.8                                                |

For comparison, we give the size of seedlings grown by conventional methods. Seeds collected at the Otradnoye BIN RAS Scientific Experimental Station (60°48'42.7"N 30°14'17.1"E) were sown in the open ground in October 2017. Measurements of seedlings were made in April 2019.
The use of new promising plant growth regulators allows to intensify the agricultural technology of *Pinus sibirica* growing from seeds, reduces the time and cost of obtaining planting material, which is not inferior in quality to the grown according to known methods.

4. Conclusions

As a result of this research, the biometrics of seeds and cones of *Pinus sibirica* from the places of natural growth and from the St. Petersburg Botanical garden of Peter the Great was studied. X-ray studies of seeds were carried out, a new method of seed propagation *Pinus sibirica*, which is absent in the literature, was developed using the original plant growth regulators. This technique greatly simplifies the technology of growing *Pinus sibirica* from seeds, does not require long-term stratification of seeds, excluded loss of seeds during autumn sowing due to eating seeds by rodents and birds. This method of cultivation is promising for nurseries of ornamental plants, landscaping, land reclamation and forestry.

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