**Eucommia ulmoides** Oliv. seeds’ germination depending on the methods of preseeding preparation

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**Abstract.** The article discusses the techniques, methods and results of *Eucommia ulmoides* Oliv. soil germination determination. Two batches of seeds collected from plants in various places of growth during introduction (Botanical gardens of Sochi and Sukhumi) were studied. The seeds’ under snow storage, stratification in a refrigerator with the subsequent sowing of seeds in open ground was carried out. After stratification of seeds from Sukhumi their soil germination increased from 11.1% to 18.5%, i.e. almost 1.7 times. It was noted that the soil germination of *Eucommia ulmoides* seeds is directly depended on their mass while the seeds’ sizes were of subordinate importance.

**1. Introduction**  
The present study is devoted to elucidation of actual and potential possibilities of seed propagation of *Eucommia ulmoides* in the conditions of St. Petersburg.  
*Eucommia ulmoides* Oliv. – elmleaved Eucommiais a monotypic species of the *Eucommiaceae* family of Garryalesorder (Garryaflower)- which is a synonym of Chinese gutta-percha tree. Based on fossils examination a few more types of *Eucommia* were described: *Eucommia constans, Eucommia eocenica, Eucommia jeffersonensis, Eucommia montana, Eucommia rolandii, Eucommia palaeoulmoides* - ancient elmleaved *Eucommia* [1]. The seeds of *Eucommia montana*, which grew in the Paleogene, are shown in Figure 1.

![Figure 1. Seeds of *Eucommia montana*, which grew in the Paleogene. The age of deposits is 48.5 million years [2].](image-url)
Some features of *Eucommia ulmoides* are described below.

*Eucommia ulmoides* is a high deciduous leaf-shedding gonochoristic tree up to 15–20 m high. Flowers of *Eucommia* are grouped either in side-flowered blossom clusters or solitary, unisexual, lacking perianth, on short pedicles. Blooming usually stretches for 40 - 50 days, female flowers being characterized by retard of growth. It sometimes results in parthenocarpy (seedless fruit formation). In the conditions of eucommia artificial propagation, some cases were noted when bisexual underdeveloped and ugly flowers appeared on male individuals [2]. The average size of the fruit is 3 - 4 cm long and 6 - 12 mm wide. When growing, pericarp forms a leathery wing-like armilla (wing of the fetus). *Eucommia ulmoides* propagates by cuttings and seeds.

Thus, contributing to the introduction of *Eucommia ulmoides* into the new habitat conditions (arboretum of St. Petersburg State Forest Technical University (SPbSFTU), we protect and preserve one of the last representatives of the genus and family.

According to the work of A L Takhtagian [3], *Eucommia ulmoides* belongs to the Holarctic Kingdom, the East Asian region and the Central Chinese province. According to Takhtagian, flora of the East Asian region is of exceptional interest both for studying the history of the development of temperate floras of the Northern hemisphere, and for solving many issues of the evolution and systematics of flowering plants [3]. The area of *Eucommia* is entirely located in China, mostly along the Yangtze river in its middle current. It looks on the map as a strip expanding to the North-West, and then to the South. It nearly reaches the border with Vietnam. *Eucommia* grows at altitudes from 300 to 2500 m, mainly in the undergrowth of subtropical mountain forests.

Currently, *Eucommia* can be already found in Europe, America, Asia, in more than ten countries and regions. This article discusses the results of *Eucommia ulmoides* seed propagation investigations, which we carried out from November 2017 to April 2019. In the history of St. Petersburg there were two attempts to introduce *Eucommia ulmoides*. The first of them was undertaken by a famous introducer E L Wolf at the beginning of the twentieth century [4]. Wolf ranked its frost resistance group as V points (according to his own scale), i.e. acrop absolutely not suitable for growing near Petrograd, with extremely short life span or dying in the first winter [4]. But ranking the frost resistance group from I to V points, Wolf at the same time elaborated: "... conventional indicators of frost resistance degree of some species in the area inevitably have a relative rather than absolute value."

The second attempt in 1978 was also unsuccessful. In the nursery of the Botanical garden (at that time – of St. Petersburg State Forest Technical Academy (SPbSFTA) this species was re-introduced by one of the authors of this article. But in the abnormally severe winter of 1978/79 (the temperature dropped below – 35°C), the seedlings froze. It is known that the *Eucommia ulmoides* can withstand a short-term decrease in temperature up to 30°C [5].

Unstable climatic conditions of the North-West of Russia create significant difficulties for the introduction of many plant species. For representatives of the Chinese flora, climate instability is particularly unfavorable. Nevertheless, the possibility of adaptation of *Eucommia ulmoides* can be evidenced by the following species accompanying it in the natural habitat and successfully growing in the Botanical gardens of SPbSFTU and BIN RAS. These are the following endemic species: *Ginkgo biloba* L., *Pseudolarix amabilis* Cord. (P. kaempferi), *Metasequoia glyptostroboides* Hu et Cheng, *Tilia tuan* Szyszsz, *Phellodendron chinense* C K Schneider. – frost resistance group V according to Wolf, *Acer davidii* Franch., bears fruit *Aser grosseri* Pax, *Rhamnus utilis* Decne. bears fruit - frost resistance group III-IV according to Wolf (with shelter II - blossoms), *Kolkwitzia amabilis* Graebn., *Sorbus koehneana* C. K. Schneider. the latter two species bloom, bear fruit and give germinating seeds (seed offspring of these species has been grown. We set the task of obtaining offspring of *Eucommia ulmoides* from seeds).

1. Replenishment of the garden collection with relict species, which is assigned the category of the status of rarity NT (near threatened) – which is in a state close to threatened.
2. The obvious usefulness of the species due to its medicinal properties (known in Chinese medicine for over 1000 years) and as a Chinese tonic.
2. Methods and Materials

2.1. The research objective
Elucidation of actual and potential opportunities of seed propagation of *Eucommia ulmoides* in the conditions of St. Petersburg and determination of prospects of its use. In passing, the expedience substantiation for *Eucommia ulmoides* introduction in St. Petersburg and its region for plants ex situ conservation (as part of collections and expositions), and also as a crop with use potential for different functional purposes was considered.

For the objectiverealization the following tasks were set:
1. To analyze the changeability of the complex of *Eucommia ulmoides* fruit morphological parameters in the process of their introduction depending on the conditions of its cultivation sites.
2. To replenish the collection of the arboretum with a new wild species of *Eucommia ulmoides*, to study its bioecological features and adaptive capabilities, to assess the viability of the taxon in the conditions of introduction in St. Petersburg along with giving recommendation of effective methods of seed propagation.
3. To analyze the changeability of *Eucommia ulmoides* fruit morphological parameters complex in the process of their introduction depending on the conditions of its cultivation sites.

2.2. Research methods
The sample of the investigated seeds was 20 seeds for each sample.

Determination of soil germination of seeds was carried out in the greenhouse and in the nursery of SPbSFTU Botanical garden. The experiment had been carried out from November 2017 to April 2019.

The study of seed germination processes was carried out according to the "Guidelines for seed production of introduced species". (SST 13056.4 – 67, SST 13056.6 – 75) [6].

The objects of the research were the seeds of *Eucommia ulmoides* introduced from 2 points of its cultivation: Sochi (Russia) and Sukhumi (Abkhazia).

Batch № 1: Seeds collected in the arboretum of Sochi (Russia) harvested in 2017, were obtained on 18.11.17, the amount was 113 seeds. In the group there grow 7 plants, their age is 55 years. The group of plants grows at an altitude of 77 m above the sea level, the exposition: SE slope, 12 degrees.

Batch № 2: Seeds collected in the arboretum of Sukhumi (Abkhazia) were obtained on 01.02.18, the amount was 180 seeds.

Seeds of *Eucommia ulmoides* were obtained from Sochi and Sukhumi on 18.11.17 and 01.02.18 correspondingly. The main data on seeds are given in Table 1.

Table 1. Morphometric parameters of *Eucommia ulmoides* seeds.

| Country/City of seed collection | 1000 seeds mass (g) | Seed length (mm) | Seed width (mm) |
|-------------------------------|---------------------|------------------|-----------------|
| Sochi - Russia                | 67±0.04             | 10.8±0.05        | 6.65±0.03       |
| Sukhumi - Abkhazia            | 75±0.03             | 16.88±0.04       | 5.88±0.03       |

From the Table it follows that among them there are the seeds from Sukhumi which have the largest linear length dimensions and the largest weight (0.075 g)/1000 seeds), as well as the greatest dimensions of the wings. The size and mass of 1 000 seeds of *Eucommia ulmoides* are the factors that are closely interrelated, but it is easier to estimate the sowing quality of seeds by their mass, which was used by the article authors in further investigations. Parameters of seeds collected in different places are given in Table 2.
Table 2. Parameters of seeds collected in different places.

| Seed collection city | Seed parameters | X     | b    | m    | t     |
|----------------------|-----------------|-------|------|------|-------|
| Sochi                | Width           | 6.65  | 1.63 | 0.36 | T_sh = 1.99 |
|                      | Length          | 10.80 | 1.11 | 0.25 |       |
|                      | Weight          | 0.0667| 0.0106| 0.0024| |
| Sukhumi             | Width           | 5.88  | 0.60 | 0.14 | T_d = 17.2 |
|                      | Length          | 16.88 | 1.10 | 0.25 |       |
|                      | Weight          | 0.0748| 0.0069| 0.0015| |

Examination of *Eucommia ulmoides* fruit from two regions of its introduction showed that its fruit are characterized by morphological heterocarpy (Figure 2).

Figure 2. *Eucommia ulmoides* morphological heterocarpy (wing fruit). Fruit in fractions from left to right: top row fruit № 1, 3, 7 – large; middle row - № 7 large fruit; bottom row № 2 – large fruit; other fruit are medium or underdeveloped.

To characterize the quality of seeds different indicators are used. The most important and commonly used is germination. Experiments on seed germination were carried out in the following variants: seed sowing was performed at three temperature regimens (in the nursery, in the greenhouse and in the refrigerator – stratification) with photo fixation. The results of the study are given in Table 3.
Table 3. Influence of seed germination conditions at different temperatures and preparation methods.

| City/country | Total number of seeds (number) | Sowing date | Total number of sown seeds (number) | Place of sowing | Method of seeds’ preparation for sowing | Number of seedlings (number) | Seeds’ germination time (month) |
|--------------|-------------------------------|-------------|-------------------------------------|----------------|----------------------------------------|-----------------------------|--------------------------------|
| Sochi        | 113                           | 21/11/17    | 45                                  | in the nursery | under snow storage                     | 3                           | 06/2018                        |
|              |                               | 28/11/17    | 45                                  | in the greenhouse | without stratification                 | 2                           | 10/2018                        |
|              |                               | 11/04/18    | 20                                  | in the greenhouse | stratification (from 28/11/17 till 10/04/18) | -                           | -                              |
| Sukhumi      | 180                           | 01/02/18    | 45                                  | in the greenhouse | without stratification                 | 5                           | 09-10/18                       |
|              |                               | 01/02/18    | 45                                  | in the nursery   | under snow storage                     | 25                          | 05-07/18                       |
|              |                               | 22.05.18    | 90                                  | in the nursery   | stratification (from 01/02/18 till 21/05/18) | -                           | -                              |

From the Table it follows that the seeds stratified in a refrigerator or subjected to undersnow storage began to germinate from the end of May, while the seeds sown directly in a greenhouse began to germinate from the end of September. Soil germination of Eucommia seeds in the mode of stratification in a refrigerator and subjected to undersnow storage is higher than soil germination of seeds planted by direct sowing in a greenhouse.

The quality of seeds is the most important characteristic of the initial stages of plant propagation and therefore this indicator should be given great attention. High quality seeds provide the starting potential for the most optimal formation of productivity and stability of plants [5-12]. The main characteristics of seeds’ quality is their germination.

In general sense, laboratory germination refers to the percentage of seeds germinated under optimal conditions. Seeds are considered to be germinated when root length equals one half the length of a seed, and for small seeds – double the length. Swollen seeds as soon as they are only increasing in size (sometimes their peel bursts), or abnormal germination of seeds, for example, in cotyledons, are taken into account separately and are not included in the calculation of germinated seeds in germination determination. A number of Eucommia germinated seeds after their stratification in sand at +5⁰ C in a refrigerator is presented in Figure 3.
Figure 3. Eucommia seeds germinated after their stratification in sand at +5 C in a refrigerator. Photodate: 21.05.18.

Determination of seed germination was carried out in a laboratory with the help of a growth test, i.e. seeds’ germination at a certain temperature and in certain light regimen in a humid medium, followed by counting germinated seeds (germination monitoring). The experiment was carried out from November 2017 to April 2019. Study of the processes of seed germination were carried out according to "Guidelines for seed production of introduced species" (SST 13056.4 – 67, SST 13056.6 – 75) [6].

Experiments on seed germination were carried out in the following variants: seed sowing was performed at three temperature regimens: in the nursery (undersnow storage), in the greenhouse (control) and in the refrigerator (stratification) with photo fixation.

In spring on 28/05/2018 sprouts’ appearance was recorded. And juvenile leaves having appeared at the end of May, seedlings singling into a bed was made in the nursery. The first measurement of all seedlings was carried out on 07.07.18 according to the following parameters: cotyledons (length), leaves (width, length), hypocotyl (length), epicotyl (length), the total height of the plant. The results of the study are given in Table 4.

Table 4. Results of biometric indicators of Eucommia ulmoides seedlings 40 days after sowing in the nursery and greenhouse.

| City where seeds were collected | Place of sowing | Total number of seeds (number) | Seeds germination (%) | hypocotyl (cm) | cotyledons (cm) | epicotyl (cm) | Total height (cm) |
|--------------------------------|----------------|-------------------------------|----------------------|---------------|----------------|--------------|------------------|
| Sukhumi                        | nursery        | 135                           | 18,5                 | 3.44          | 3.79           | 0.99         | 4.87             |
|                                | greenhouse     | 45                            | 11,1                 | 4.1           | 3.88           | 0.85         | 5.58             |
| Sochi                          | nursery        | 45                            | 6,7                  | 4.0           | 3.90           | 1.10         | 5.70             |
|                                | greenhouse     | 45                            | 4,4                  | 3.6           | 3.35           | 0.53         | 4.37             |

There was observed an interesting special feature in the appearance of juvenile leaves of *Eucommia ulmoides* – they were located opposite, while the subsequent leaves were morphologically similar to
juvenile leaves and were arranged alternately. The dependence of seed germination on their mass is shown in Table 5.

Table 5. Dependence of soil germination of *Eucommia ulmoides* seeds on the mass of 1000 seeds

| City where seeds were collected | Place of sowing    | Mass of 1000 seeds (gr) | Germination (%) |
|---------------------------------|--------------------|-------------------------|-----------------|
| Sukhumi nursery greenhouse      | 75                 | 18.5                    | 11.1            |
| Sochi nursery greenhouse        | 67                 | 6.7                     | 4.4             |

From the data in Table 5 it can be seen that the germination of *Eucommia ulmoides* seeds depends on the initial seed mass. Seeds from Sochi with a mass of 67 g have low germination rate (4.4–6.7%), while germination of seeds from Sukhumi is higher: it can reach up to 11.1–18.5 per cent. The best qualities have seeds collected in the Botanical garden of Sukhumi (11.1–18.5%). This is due to their formation in optimal environmental conditions.

Due to the unstable climatic conditions of St. Petersburg in the autumn-winter-spring period, it was necessary to guarantee the preservation of all seedlings of *Eucommia ulmoides*. To preserve the plants, half of the seedlings was transplanted into pots and placed in a greenhouse. After digging seedlings out on 12.10.18 their measurements were made: the largest diameter of roots, the length of roots and the total height of the seedlings. The results of measurements of *Eucommia ulmoides* seedlings are presented in Table 6.

Seedlings left in the open ground were covered with a double layer of spunbond on 30.11.18.

Table 6. Results of 4–month old *Eucommia ulmoides* seedlings parameter measurements.

| Maximum root diameter (mm) | Maximum root length (cm) | Total height of seedlings (cm) |
|----------------------------|--------------------------|--------------------------------|
| 1                          | 1.3                      | 6.2                            | 11.1                           |
| 2                          | 1.1                      | 5.6                            | 9.3                            |
| 3                          | 1.5                      | 7.8                            | 7.6                            |
| 4                          | 1.0                      | 6.6                            | 7.4                            |
| 5                          | 0.8                      | 6.5                            | 7.0                            |
| 6                          | 0.9                      | 4.1                            | 6.9                            |
| 7                          | 1.6                      | 5.6                            | 10.7                           |
| 8                          | 1.2                      | 9.7                            | 9.2                            |
| 9                          | 0.8                      | 7.0                            | 6.5                            |
| 10                         | 1.5                      | 9.2                            | 5.1                            |
| 11                         | 0.9                      | 5.0                            | 5.3                            |
| 12                         | 0.9                      | 6.1                            | 10.6                           |
| **X**                      | **1.1**                  | **6.6**                        | **8.1**                        |

The maximum root length of 4-month-old seedlings is 9.7 cm. The minimum root length is 4.1 cm. The average length of the roots is 6.6 cm. The sprouts do not have root hair on the root endings, which may indicate the presence of mycorrhiza.
3. Conclusions
1. Morphological parameters of seeds of *Eucommia ulmoides* from two of its introduction points are as follows:
   - the average length of a seed: Sochi - 10.8 mm;
   - average seed width: Sochi – 6.65 mm;
   - mass of 1000 seeds: Sochi – 67 g;
   - the average length of a seed: Sukhumi – of 16.88 mm;
   - average seed width: Sukhumi – 5.88 mm;
   - mass of 1000 seeds: Sukhumi – 75 g
2. Indicators of soil germination of *Eucommia ulmoides* seeds without stratification are low – Sochi- 4.4%; Sukhumi – 11.1%.
3. The necessity of cold stratification is confirmed. After stratification of seeds from Sukhumi within 3 months, the soil germination increased up to 18.5%, i.e. almost 1.7 times;
   - seeds subjected to cold stratification had higher germination rates and shorter germination terms than those sown directly in a greenhouse;
   - seeds subjected to cold stratification begin to germinate from the end of May till the middle of July.
   - control seeds (sown in a greenhouse) began to germinate from the end of September 2018 till the middle of January 2019.
4. The propagation and quality of seeds of *Eucommia ulmoides* are greatly influenced by the species gonochorism. Female flowers female flowers are characterized by retard of development (protandry). Flowering of female flowers stretches and lasts as many as 45-50 days, which contributes to the formation of seedless fruit (parthenocarpy).
5. Despite the identical climatic conditions of Sochi and Sukhumi, seeds from Sukhumi harvested in 2017 turned to be more suitable from the point of view of obtaining seeds of local propagation to obtain offspring from them. This is evidenced by the lower mass of 1000 seeds from Sochi (67±0.04 mGy) and a high share - 96.5% - of parthenocarpic fruit or underdeveloped seeds. Mass of 1000 seeds from Sukhumi is (75 ±0.03 g) and the percentage of parthenocarpic fruit is lower - 88.3 %.
6. Seeds of *Eucommia ulmoides* from the city of Sukhumi harvested in 2017 are characterized by the best sowing qualities, which proves the necessity of clearer determination of the geographical points of seed collection for primary introduction.
7. There is observed a significant number of parthenocarpic fruit of *Eucommia ulmoides* from the two points, which confirms the need for research to determine the pollen fertility of the species.
8. It should be noted that the number of parthenocarpic fruit in the studied batches varies from 81.5% of the (Sukhumi) to 93.3% (Sochi) that can be caused by *Eucommia ulmoides* species gonochorism, insufficient quantity of pollen during pollination, as well as its different quality.

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