A RESEARCH ON SEED PROPAGATION OF *Cunninghamia konishii* HAYATA AT THE NURSERY

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1. INTRODUCTION

Seed germination and seedling survival in forest habitats are critical for natural regeneration, plant propagation, and seed dispersal. Many seeds are produced and dispersed, but most of them are not germinate or survive after germination. The mortality of seeds and seedlings is related to the species, natural regenerative potential. It is associated with the genetic determination of future individuals. Several studies have shown that seed germination, seedling development, and survival rate are all influenced by various internal and external environmental factors, all are considered species-specific [1, 2]. Many plants have been identified as fast-growing and high-yielding plants, whereas many seed plants have hard seed coats that prevent them from germinating in normal conditions [3]. As a result, studies on the factors influencing germination, survival, and seedling growth for a specific plant species are expected.

Cunninghamia konishii is a tall, evergreen tree with a trunk diameter of 2-3.5 m that belongs to the Cupressaceae [4, 5]. This species is only found in Laos, China, and Taiwan. However, they are extremely rare in Vietnam, occurring only in Ha Giang (Hoang Su Phi and Tay Con Linh), Son La (Xuan Nha), Thanh Hoa (Xuan Lien), and mainly Nghe An (Ky Son and Que Phong districts. Quy Hop, Con Cuong, on the Vietnam-Laos border mountain range) [6, 7]. At an elevation of 960 - 2000 m above sea level, a dense primeval evergreen mixed tropical monsoon low mountain or medium mountain forest (average annual temperature 13-19ºC, total annual rainfall above 1500 mm) grows on soil weathering from granite or other silicate primary rocks [8-11]. These species have attractive veins and hues, and the bark trees release oil resin used as medicine, therefore they have a high use value [11]. Moreover, they are also used as scenic plants in parks [12, 13]. As a result, natural regeneration is limited, resulting in a small number of individuals, small natural populations, and overexploitation, which exposes species at the risk of extinction [12]. The International Union for Conservation of Nature (IUCN) categorizes species as endangered (EN) in 2013 [12,13], Red Book of Vietnam (2007) classed them as vulnerable (VU) [11]. Vietnamese Government's Decree No. 06/2019/ND-CP listed this species in endangered and uncommon forest plants (IIA group) [14], barred from extraction and use for commercial purposes. The main danger with the natural population of the species, where natural forest reserves have been systematically and considerably diminished, without the help of a regeneration strategy [9, 10]. This is due to the presence of significant geographical obstacles preventing genetic variation between these population of the species. As a result, plant propagation should be a main challenge, particularly for *C. konishii*, and seedling propagation should be a part of our culture in order to produce a suitable environment.

This study focused on studying seed germination, seed survival, and seedling growth in the nursery stage under different artificial environmental conditions to provide the best growth opportunities. Therefore, this study has been a scientific-based for the breeding, conservation, and development programs this species.

2. MATERIALS AND RESEARCH METHODS

2.1. Material

We had collected seeds of *C. konishii* from Pu Hoat Nature Reserve, Nghe An province. All seeds used in this study were collected during the period from early November to early December 2020. Only seeds of the mature fruits with a dark brown fruit coat were collected. The mature fruits were dry-stored immediately after collection and transferred to the Vietnam - Russia Tropical Centre. In propagation nursery: Nursery soil that has had its topsoil scrapped and rebuilt appropriately. Usually, it is taken in the B layer of the soil profile, and the distance to the surface layer is 40 cm. Then, it was putted in plastic planters; Manure and superphosphate in various proportions.

2.2. Methods

(-) Testing the purity of seeds:

Particle purity: The purity of grain is assessed by the percentage difference between pure seeds weight and the test sample weight, which is calculated through the use of three test samples and the following process:

- Weigh the weight of three samples with an accuracy of 10^{-3} grams. In which, the test sample weight is 3000 seeds equivalent to 15g. Divide the test sample into three sections: good seeds (firm seeds that are not damaged); discarded seeds (broken seeds, diseased seeds, too tiny seeds, flat seeds); and impurities (broken seeds, diseased seeds, too tiny seeds, flat seeds) (gravel, sand, debris, other tree seeds...).

Figure 1. Full-grown plant (A), branches bearing fresh cones (B), dried cones (C), seeds (D)

The following formula can be used to determine the purity of a seed sample:

$$\text{Purity of seeds (\%)} = \frac{\text{Pure seeds weight(g)}}{\text{Test sample weight}} \text{x } 100 \quad (1)$$

Note: Pure seed means seed free of inert matter and free of other seed distinguishable by appearance or by test.

(-) Effects of various conditions on seed germination:

All seeds used in this experiment were fresh seeds that had been left to dry naturally within 7 days after collection. The seeds were surface disinfected by soaking them in a solution of 0.1% KMnO4 potassium permanganate for 30 minutes to remove the acidity and were washed with cool water before being sown. A total of 900 seeds were equally distributed to three different treatments (CT) (300 seeds per treatment).

CT1: Soak seeds in water at room temperature for 8 hours, then drop seeds in moist sand;

CT2: Soak seeds in 40ºC water for 8 hours (to allow them to stay cool constantly), then plant the seeds in moist sand;

CT2: Soak seeds in 60ºC water for 8 hours (to allow them to stay cool constantly), then plant the seeds in moist sand;

Determine the germination rate of seeds using formular (2):

$$\text{Germination percentage (\%)} = \frac{\text{Seeds germinated}}{\text{Total seeds}} \text{x } 100 \quad (2)$$

Determine the speed of germination using formular (3):

$$\text{Speed germination (\%)} = \frac{\text{Number of germinating seed in } \frac{1}{3} \text{ of germinating time}}{\text{Total germinating seeds}} \text{x } 100 \quad (3)$$

(-) Effect of composition of the potting soil on the growth of seedlings:

The following ingredients are used in the potting soil mixture for growing seedlings according to the potting density: nursery soil (top soil), manure, and superphosphate in the following experiment proportions:

CT1: consits of 98% soil and 2% superphosphate.

CT2: consists of 88% soil, 10% farmyard manure, and 2% superphosphate.

CT3: consists of 78% soil, 20% farmyard manure, 2% superphosphate.

Each experiment (CT) contains 100 plants that are arranged in a randomly. The seedlings used in the tests were strongly predictive of growth and quality at the time of feeding.

Monitor plant growth up to 4 months of age. The monitoring indicators are height (Hvn), root diameter (D_{00}), tree quality according to classification A, B, C. In which, A: good growth, straight stem, no pests; B: medium growth, insignificant levels of problems; C: poor growth, pests, shriveled plants…

(-) Effect of shading on the growth of seedlings:

The experiment was conducted from January 2021 to December 2021, at the *Vietnam National University of Forestry*, Xuan Mai town, Chuong My district, Ha Noi.

Evaluation effect of shading regime on seedlings (using Plastic shade netting) conducted through the following experimental formulas:

CT1 - no shading

CT2 - 25% shading

CT3 - 50% shading

CT4 - 75% light shading.

100 plants per treatment, randomly arranged. Monitor plant growth up to 4 months of age. The monitoring criteria are height (Hvn), root diameter (D_{00}), tree quality according to classification A, B, C (good, medium, and bad).

(-) Data analysis:

Collect seed germination data: Beginning at the time the seeds began to germinate, keep track of the number of seeds that have germinated at CT on a regular basis until the seeds have finished germination. The final date of germination was the day after which 5 days later the number of seeds germinated by no more than 5%.

Obtaining information about the development of seedlings: Every 20 days, starting from the moment the plants are transplanted into the pot, data on the survival rate of the plants and the survival rate of diseased plants is collected. Keep records of growth parameters such as height, diameter, and the number of leaves.

Use sample analysis and variance analysis to process and analyze data. SPSS 20.0 and Excel software were used. One-factor ANOVA analysis was used to study the differences in relative growth traits between different treatments. The difference is considered significant if the *P*-value of *F* is less than 0.05.

3. RESULT AND DISCUSSION

3.1. The effect of treatment methods on seed germination

Seed lot purity: There were also significant differences in mean purity between certain treatment combinations (Table 1). The results of determining the average purity of the 3 CT experiments of seeds tested were 70%, of which: CT1 had the highest purity of 75.7%, followed by CT2 (68.9%), CT3 (62.5%). In order to demonstrate the quality of seeds that were seeded after being taken from the wild was uniform, the number of poor quality seeds in each experiment is averaged across all. In order to demonstrate the quality of seeds that were seeded after being taken from the wild was uniform, the number of poor quality seeds in each experiment is averaged across all [15]. The results of this study were similar to those of *Pinus tropicalis*, which showed a common seeds yield of 40 671±272 seeds/kg with healthy seeds (60.5%±1.0%), empty seeds (7.2%±0.4%) and infected seeds (2.3%±1.0%) [11].

Figure 2. Dry cones (A), tailings (B), good seed grain (C)

Seed germination capacity: The germination process of seeds according to three different treatment methodologies has a lengthy different germination period. After 10 days, seeds in the experimental plot CT3 began germinating, followed by seeds in the CT1 and CT2 (after 15 days), although seeds treated at air temperature in CT1 were the quickest to finish germination (after 10 days). Table 1 shows the germination capacity of seeds after being given to different treatments.

According to the results in Table 1, the germination rate of seeds in the tests ranged from 7.3 to 30.3%. Seeds treated at 60^{o}C (CT3) had the greatest germination rate (30.33%), followed by seeds soaked in water at 40^{o}C (CT2), which had a germination rate of 22%, and seeds treated with water (CT1) in normal air temperature reached 7.3%. However, through over 40 days of surveillance, the results revealed that some seeds germinated but eventually decayed and dead the roots. The quantity of germinated seeds and seedlings declined after 50 days.

Table 1. Seed germination capacity of *C. konishii*

	Total number of seeds germinated							**Germination rate (%)**	**Speed germination (%)**
	Number of seed	**10 days**	**15 days**	**20 days**	**25 days**	**30 days**	**35 days**		
CT1	300	0	5	10	13	19	22	7,3	1,7
CT2	300	0	17	27	38	45	66	22,0	5,7
CT3	300	7	22	37	57	65	91	30,3	7,3

Some studies have been conducted on seed germination in Cupressaceae [17-19]. Germination was improved by soaking seeds in potassium permanganate ($KMnO_4$), concentration 0.05%, for 10 minutes before planting seeds, then selecting out, rinsing, and soaking in pure water for 1-2 hours, plant the seeds on gourd with sand mixed with a little soil, then covering the seeds with a thin layer of soil,

attempting to cover the comforter with fibre, and using Plastic shade netting to shade the sun. Water are on an order to keep the humidity at a comfortable level. The seeds germinated with a success rate of 23% after 30-40 days [17]. It also were recently gathered cones from damaged branches, dried them, removed the seeds from the cones, and choosed strong seeds. After one month, plant seeds in sand, remove stones and treat with Vi ben C 0.25% (2.5g/1 liter) before 6-7 days ago. After 10 minutes of soaking in 0.05% $KMnO_4$ solution, remove the seeds, wash them, and soak them in 40ºC (2 boiling 3 cold) water, then nurture the seeds in a cloth bag for 48 hours. This result revealed that after 45 days, the successful germination rate of seeds was 22.5%. This result indicated that the seeds were treated before to planting by soaking in 0.1% $KMnO_4$ potassium permanganate solution for 30 minutes, then rinsed and soaked in water at 60ºC for 8 hours (to gradually cool down), before planting in moist sand. The germination rate of seeds after 35 days was (30.3%). In comparison, higher levels of seed germination have been reported in *Fokienia hodginsii* (48%) [18] and *Taxodium distichum* (50%) [19]. Our results demonstrate that when the temperature of the treatment water is increased or lowered, the germination rate of seeds increases and decreases significantly. Furthermore, because its seeds contain a lot of oil, new cones must be dried in the shadow to separate the seeds after cutting. Seeds should not be stored under normal conditions for an extended period of time since this will limit seed germination.

Furthermore, the seed germination rate differed slightly between treatments. As a result, CT3 has the highest germination (7.3%), followed by CT2 and CT1 with 5.7% and 1.7%, respectively. Thus, it can be shown that seeds treated by soaking in warm water at 60ºC for 8 hours and then planting on moist sand had a higher germination rate and speed than seeds treated by soaking in 40^{o}C warm water and normal water.

3.2. Effect of potting soil composition on survival and growth of seedlings in the nursery

The mean survival rate, quality, and growth characteristics (height, diameter) of the plants at the age of 4 months (Table 2). Diameter and height of seedlings were considerably differed between experimental treatments. CT2 (88% soil + 10% farmyard manure + 2% superphosphate) produced the best results in terms of height and diameter (4.7 cm and 1.0 mm), followed by CT3 (78% soil + 20% farmyard manure + 2% superphosphate) with an average height of 4.1 cm and an average root diameter of 0.8 mm, and CT1 (98% soil + 2% superphosphate). The findings of a one-factor analysis ANOVA of variance revealed that the composition of potting had a significant influence on seedling growth at the nursery stage ($F=50.7 > F_{crit}=3.2$) with $P < 0,05$. The potting solution CT2 produced the greatest growth results in diameter and height of plants.

Table 2. Effect of potting soil composition on growth of seedlings in the nursery

	Survival plants	Rate (%)	Growth criteria		Qualities of plants (%)		
			Hvn (cm)	D_{00} (mm)	A	B	C
CT1	75	75	3,7 ±0,4	0,7±0,07	67	22	11
CT2	91	91	4,7±0,4	1,0±0,96	75	21	4
CT3	83	83	4,1±0,2	0,8±0,05	72	15	13

3.3. Effect of a shade system on the development of 4-month-old plants

The monitoring the growth characteristics of seedlings at 4 months of age under different shading treatments revealed that plant height varied with varying amounts of shade (Table 3). The tree height reached the highest value in the 25% shade formula (4.7cm), followed by the no shading method. The height in the 75 percent shading formula is just 3.6 cm, which is lower than that in the 50 percent shading calculation (4.1 cm). The height of plants under different shade treatments was substantially different (F = 96.8 > Fcrit = 2.72) with P-value 0.05 when the difference between the experimental formulae was checked using one-factor analysis of variance. The root diameter factor is most significant in the 25% shade formula (1.1 mm). Gradually declines in the no shading (0.9 mm) formula, 50% shading (0.8 mm) formula, and lowest in the 75% shading calculation (0.7 mm). The degree of shade had a significant influence on the root diameter of the seedlings (F = 43.3 > F crit=2.72) with a P-value of 0.05 when the difference between the experimental formulae was examined using single-factor analysis of variance.

The survival rate of seedlings in the shade experiment conditions was greater than 85% of total experimental seedling plants. The formula with 25% shade and 0% shading provided the most quality plants (87%). This demonstrates that shadow substantially influences the growth criteria of seedlings at the nursery stage in terms of height, root diameter, and quality. In general, while studying plant nurseries, investigators focus on determining the ecological variables that significantly impact the growth of seedlings, with light seems to be of particular importance. When planning the experiment on the function of the shading method, it was split into five categories of shading: no shading, 25% shading, 50% shading, 75% shading, and 100% shading. Effect of shade percentage on the growth of seedlings in the nursery stage revealed that: at the age of 1-4 months, 25% shading is guaranteed for seedlings. The monitoring data (from each stage till the plants are 4 months old) clearly demonstrated the results. The biomass and height of plants increased substantially.

Table 3. Effect of a shade system on the development of 4-month-old plants

	Survival plants	Rate (%)	Qualities of plants (%)			Growth criteria	
			A	B	C	Hvn (cm)	D_{00}(mm)
No Shading	95	95	87	9	4	4,3±0,2	0,9±0,1
Shading 25%	96	96	87	11	2	4,7±0,3	1,1±0,1
Shading 50%	95	95	73	20	7	4,1±0,2	0,8±0,1
Shading 75%	85	85	65	22	13	3,6±0,2	0,7±0,1

4. CONCLUSION

The results revealed that the evaluated seeds had an average purity of 70.3%, with seeds soaked in warm water at 60ºC having the most remarkable germination rate of 30.33%. The potting mix contains 88% soil, 10% farmyard manure, and 2% superphosphate (CT2) for the most significant growth. The 25% shading formula is optimum for the growth of plants in the nursery period up to 4 months of age, with a survival rate of 96%. The information presented above is significant scientific evidence that will assist in the design of a future solution for this species' germination issues.

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SUMMARY

A RESEARCH ON SEED PROPAGATION OF *Cunninghamia konishii* HAYATA AT THE NURSERY

Cunninghamia konishii Hayata has not only ecological importance in the forest ecosystem but also great commercial value in timber and fragrant essential oil. Them listed it as endangered in Red Book of Vietnam and IUCN Red List. This species naturally grows at an altitude of 1,200-1,600 m on the humus alpine and species also lives together with other species conifer such as *Fokienia hodginsii*, *Amentotaxus argotaenia* and *Podocarpus* spp.,… *C. konishii* was poor regeneration status. The paper presents the results of seed propagation and growth of *C. konishii* at the nursery stage. The result shows that the seed purity was 70.3% after 35 days. Seeds were treated and soaked in warm water at 60^{o}C for the highest germination rate of 30.3%. Seedlings grew quickly and evently in a potting mix of 10% muck and 2% superphosphate. The formula of 25% shading for the growth of *C. konishii* in the nursery period up to 4 months of age is the best with a survival rate of 96%, growth height was 4.7 cm, diameter was 1.1mm. Our study will assist conservators in future conservation management, breeding, production and habitats restoration of the species.

Keywords: *Cunninghamia konishii, germination, habitats, seeds, nursery.*

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