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# Standardization of pre-sowing treatments for seed germination and seedling growth of *Ginkgo biloba* L. under temperate conditions of Kashmir Himalayas

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## Abstract
The present investigation entitled "Standardization of pre-sowing treatment for seed germination and seedling growth of *Ginkgo biloba* L. under temperate conditions of Kashmir Himalayas was carried out at experimental field of Faculty of Forestry, Benihama, Ganderbal Jammu and Kashmir. Pre-sowing treatments viz, Stratification, Scarification and soaking seeds in water for 24 hours to stimulate the germination of seeds and early establishment of their seedlings revealed that 60 days stratification at 4±1ºC have shown better results in terms of germination percentage (80.91%), germination energy (19.67). Percent increment in terms of seedling height (68.85%), collar diameter (17.49%), leaf area (63.92%), total fresh biomass (63.28 %) and total dry biomass (59.83 %) respectively. Therefore in order to obtain better seed germination and seedling growth, the seeds of *Ginkgo biloba* should be stratified for 60 days at 4±1ºC before sowing.

**Keywords:** pre-sowing treatment, *Ginkgo biloba*, Kashmir, stratification

## Introduction
The state of Jammu and Kashmir lies in Western Himalayas which has been recognized as floristically under explored by the Botanical Survey of India (Dar *et al*., 2012) [8]. Jammu and Kashmir is a hilly state with an area of 2,22,236 Km$^2$ (Anonymous, 2015) [2]. Biogeographically, it comprises of three district provinces: the subtropical Jammu, the predominantly temperate Kashmir, and the cold-arid Ladakh. About 2/3$^{rd}$ of the state's total area is recorded under forest and substantial part of this is non-conducive for the growth, being under permanent snow, glaciers and cold deserts (Anonymous, 2015) [2]. *Ginkgo biloba* L. the sole survivor of the ancient family of Ginkgoaceae is the world's oldest tree. Also known as Maidenhair tree, Ginkgo is a monotypic genus native to China (Bailey, 1923) [3]. *Ginkgo biloba* was admitted as a living fossil of Jurassic period that emphasizes its relic position (Jacobs and Browner, 2000) [14]. *Ginkgo biloba*, which is not closely related to any other living plant, is generally classified in its own division the Ginkgophyta. Ginkgo is a hardy tree, tolerating a variety of climate and soil types. It can grow well in climates with a mean annual temperature from 8 to 20℃ and can tolerate a minimum temperature of -20℃ and annual rainfall ranging from 840 to 1400mm. The tree can survive for a short period at seasonal extreme temperature of 40℃ and -30℃ in China (Cao, 2003; Cheng, 1993) [6, 7]. The Ginkgo tree is grown in many regions of the world, now cultivated extensively in Asia, Europe, North America, New Zealand and Argentina.

The species is cultivated commercially for obtaining leaves which are known to contain a wide variety of medicinally active chemicals, most notably terpinoides and flavonoides (Boralle *et al.*, 1988; Defeudis *et al.*, 1998) [5, 9]. Ginkgo leaf extracts are used for many pharmaceutical purposes. The leaves of this species are extensively used in the form of a concentrated standardized *Ginkgo biloba* Extract (GBE) in different countries of the world (particularly in China, Europe, France and Germany) as a source of herbal medicine. This extract is taken internally for the treatment of cerebral and peripheral vascular diseases, as well as to alleviate some of the ailments associated with ageing, including dizziness, ringing in the ears and short term memory loss deterioration.

*Ginkgo biloba* L. was listed as a rare species in the IUCN 1997 red list of threatened plants and listed in the red list of endangered plant species. Because of it is high medicinal value the species has been exploited by pharmaceutical industries all over the world. The demand for *Ginkgo biloba* in the world is increasing from 26-36% every year. So, there is urgent need to cultivate *Ginkgo biloba* at large scale (Masood, 1997) [17]. The species is sparely distributed in some parks of Kashmir valley and till date no concrete efforts have been made for *ex-situ* conservation of this species of highest medicinal value.

There are so many factors which cause dormancy including seed coat, embryo or inhibitors which influence the seed germination rate (Agarwal and Dadlani, 1995) [1]. Many methods are used to overcome these problems depending on the type of plant species and dormancy. Such methods include heating (Herranz *et al*., 1999) [13], stratification, scarification (Narbona *et al*., 2003) [20] and gibberellins application. Scanty work has been done to elucidate the influence of stratification of Ginkgo seeds which often facilitates after ripening of embryo and germination ability (Ponder *et al*., 1981; Willan, 1985) [21, 26]. The seeds of *Ginkgo biloba* exhibit both physical as well as physiological dormancy. Moist chilling or cold stratification is widely used for breaking seed dormancy and increasing the rate of germination percentage of dormant seeds of many species (Wang and Berjak, 2000) [28].

## Materials and Methods

The present study was conducted in the Faculty of Forestry, SKUAST-Kashmir, Benhama, Ganderbal, J&K. The site lies on the southern aspect at $34^0$16'44"N and $74^0$46'31"E. The study area is located at an elevation of 1783m (5850 feet) above the mean sea level. The study area has temperate climate experiencing four distinct seasons: a severe winter (December to Febuary), a cold spring (March to May), a mild summer (June to August) and a pleasant autumn (September to November). The site falls in a mid to high altitude characterized by hot summer and very cold winters. The average precipitation is 690 mm most of which is received from December to April in the form of snow and rains

The seeds of *Ginkgo biloba* were collected from available sites in the month of October-November. The outer fleshy coat (sarcotesta) was removed and the seeds were washed in water and then air dried at room temperature and stored. The seeds were stratified at 15 days interval for 90 days at a temperature of $4\pm1$ $^0$C. Besides this seeds were also subjected to scarification treatment and soaking in water for 24 hours before sowing in polybags.

## Results and Discussion

### Germination parameters

It is evident from table 1 that germination percentage increased with increase in the stratification duration. The maximum germination percentage 80.91% was recorded in the seeds stratified for 60 days. The scarified seeds showed germination percentage of 65.89% and that of water soaked seeds produced germination percentage of 55.90%. The minimum germination percentage of 28.47% respectively was recorded in the seeds under control. All other treatments were statistically significant ($p\leq0.05$) with each other. The results on germination energy of *Gingko biloba* seeds had shown increasing trend with increase in the stratification duration. The maximum germination energy of 19.67 was recorded in the seeds stratified for 60 days. The scarified seeds exhibit germination energy of 13.33 and that of water soaked seeds produced germination energy of 10.67. The minimum germination energy of and 8.33 was recorded in the seeds under control. All other treatments were statistically significant ($p\leq0.05$) with each other.

### Seedling growth

The perusal of data in Table-2 revealed that seedling height of *Ginkgo biloba* increased with increase in stratification duration. The seedlings raised from seeds stratified for 60 days exhibited maximum plant height growth of 17.00cm with an annual increment of 68.85% at the end of second growing season. The seedlings raised from scarified seeds exhibited plant height growth of 12.20cm with an annual increment of 53.82% for second growing season and seedlings raised from water soaked seeds produced plant height growth of 11.87cm for the first growing seasons with an annual increment of 33.69% at the end of second growing season. The minimum height growth of 9.05cm with annual increment of 14.94% was recorded for seedlings raised from seeds that were not subjected to any treatment (control).

**Table 1:** Effect of pre-sowing treatments on germination (%) and germination energy of *Ginkgo biloba* seedlings.

| | Treatments | Germination (%) | Germination Energy |
|---|---|---|---|
| Control | $T_1$ | 28.47 | 8.33 |
| Stratification | $T_2$(15 days) | 42.78 | 10.33 |
| | $T_3$(30 days) | 56.11 | 12.33 |
| | $T_4$(45 days) | 69.54 | 15.00 |
| | $T_5$(60 days) | 80.91 | 19.67 |
| | $T_6$(75 days) | 77.32 | 17.00 |
| | $T_7$(90 days) | 73.54 | 15.00 |
| Scarification | $T_8$ | 65.89 | 13.33 |
| Water soaked | $T_9$ | 55.90 | 10.67 |
| | Mean | 61.16 | 13.57 |
| | CD($p\leq5$) | 5.31 | 3.31 |

**Table 2:** Effect of pre-sowing treatments on seedling characteristics of *Ginkgo biloba*.

| | Treatments | Seedling height (cm) | | | Collar diameter (mm) | | | Leaf area/plant ($cm^2$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2016 | 2017 | Increment (%) | 2016 | 2017 | Increment (%) | 2016 | 2017 | Increment (%) |
| Control | $T_1$ | 9.05 | 10.64 | 14.94 | 5.40 | 5.73 | 5.76 | 72.75 | 90.43 | 19.55 |
| Stratification | $T_2$(15 days) | 12.34 | 17.84 | 30.83 | 7.50 | 8.20 | 8.54 | 164.06 | 222.33 | 26.21 |
| | $T_3$(30 days) | 12.56 | 33.36 | 62.35 | 8.60 | 9.50 | 9.47 | 211.47 | 534.41 | 60.43 |
| | $T_4$(45 days) | 16.48 | 50.86 | 67.60 | 9.20 | 10.22 | 9.98 | 248.25 | 666.91 | 62.78 |
| | $T_5$(60 days) | 17.00 | 54.58 | 68.85 | 10.28 | 12.46 | 17.49 | 286.28 | 793.42 | 63.92 |
| | $T_6$(75 days) | 16.00 | 46.61 | 65.67 | 8.74 | 10.50 | 16.76 | 250.04 | 583.25 | 57.13 |
| | $T_7$(90 days) | 14.07 | 36.63 | 61.59 | 8.53 | 9.80 | 12.96 | 215.65 | 454.81 | 52.58 |
| Scarification | $T_8$ | 12.20 | 26.42 | 53.82 | 7.50 | 8.47 | 11.45 | 170.97 | 336.69 | 49.22 |
| Water soaked | $T_9$ | 11.87 | 17.90 | 33.69 | 7.34 | 8.08 | 9.16 | 128.69 | 190.72 | 32.52 |
| | Mean | 13.51 | 32.76 | | 8.12 | 9.22 | | 194.24 | 430.33 | |
| | CD($p\leq5$) | 2.38 | 4.89 | | 0.94 | 1.24 | | 43.87 | 65.78 | |

The data presented in Table-2 revealed that All the treatments were statistically significant at ($p\leq0.05$) with each other. The results recorded show that collar diameter of *Ginkgo biloba* increased with increase in stratification duration. The seedlings raised from seeds stratified for 60 days exhibited maximum diameter growth of 10.28mm with annual increment of 17.49% at the end of second growing season. The seedlings raised from scarified seeds exhibited diameter growth of 7.50mm with annual increment of 11.45% at the end of second growing season and seedlings raised from water soaked seeds produced diameter growth of 7.34mm with annual increment of 9.16% at the end of second growing season. The minimum diameter growth of 5.40mm with annual increment 5.76% was recorded for seedlings raise from seeds that were not subjected to any treatment (control).

The data presented in Table-2 depicts that the maximum leaf area of 286.28 $cm^2$ $plant^{-1}$ was recorded for seedlings raised from seeds stratified for a period of 60 days during the first and second growing season with an annual increment of 63.92%. The seedlings raised from scarified seeds exhibited leaf area of 170.97$cm^2$ $plant^{-1}$ for the first growing seasons with an annual increment of 49.22% and seedlings raised from water soaked seeds produced leaf area of 128.69 $cm^2$ $plant^{-1}$ for the first growing season with an annual increment of 32.52%. The minimum leaf area of 72.75$cm^2$ $plant^{-1}$ for the first growing season with an annual increment of 19.55% was recorded in the seedlings raised from seeds that were germinated without any treatment (control).

The perusal of data in Table-3 depicts that the maximum total fresh biomass of 18.86g during first year with annual increment of 63.28% at the end of second growing season was recorded in the seedlings raised from seeds stratified for 60 days stratification. The seedlings raised from scarified seeds exhibited total fresh biomass of 12.61g for the first growing season with an annual increment of 53.64% at the end of second growing season and seedlings raised from water soaked seeds produced total fresh biomass of 6.96g for the first growing season with an annual increment of 51.75% at the end of second growing season. The minimum total fresh biomass of 4.48g for the first growing season with an annual increment of 39.29% at the end of second growing season was recorded in the seedlings raised from seeds that were not subjected to any treatment (control).

The perusal of data in Table-3 depicts that the maximum total dry biomass of 9.42g during first year with annual increment of 59.85% at the end of second growing season was recorded in the seedlings raised from seeds stratified for 60 days stratification. The seedlings raised from scarified seeds exhibited total dry biomass of 5.50g for the first growing season with an annual increment of 40.86% at the end of second growing season and seedlings raised from water soaked seeds produced total dry biomass of 3.34g for the first growing season with an annual increment of 27.86% at the end of second growing season. The minimum total dry biomass of 2.22g for the first growing season with an annual increment of 8.64% at the end of second growing season was recorded in the seedlings raised from seeds that were not subjected to any treatment (control).

The data in Table-4 shows that the maximum shoot root ratio of 1.56 and 1.95 during first and second growing season was recorded in the seedlings raised from seeds stratified for 60 days stratification. The seedlings raised from scarified seeds exhibited shoot root ratio of 0.84 and 1.27 for the first and second growing seasons respectively and seedlings raised from water soaked seeds produced shoot root ratio of 0.80 and 1.03 for the first and second growing seasons respectively. The minimum shoot root ratio of 0.63 and 0.71 for the first and second growing seasons was recorded in the seedlings raised from seeds that were not subjected to any treatment (control).

**Table 3:** Effect of pre-sowing treatments on total fresh and dry biomass of *Ginkgo biloba* seedlings.

| | Treatments | Total fresh biomass(g) | | | Total dry biomass(g) | | |
|---|---|---|---|---|---|---|---|
| | | 2016 | 2017 | Increment (%) | 2016 | 2017 | Increment (%) |
| Control | $T_1$ | 4.48 | 7.38 | 39.29 | 2.22 | 2.43 | 8.64 |
| Stratification | $T_2$(15 days) | 12.54 | 25.40 | 50.63 | 4.25 | 5.84 | 27.23 |
| | $T_3$(30 days) | 15.70 | 36.26 | 56.70 | 6.58 | 13.01 | 49.38 |
| | $T_4$(45 days) | 17.91 | 46.40 | 61.40 | 7.94 | 18.58 | 57.26 |
| | $T_5$(60 days) | 18.86 | 51.37 | 63.28 | 9.42 | 23.45 | 59.83 |
| | $T_6$(75 days) | 15.48 | 39.87 | 61.17 | 7.25 | 16.40 | 55.79 |
| | $T_7$(90 days) | 14.72 | 35.00 | 57.94 | 6.26 | 13.17 | 52.47 |
| Scarification | $T_8$ | 12.61 | 27.20 | 53.64 | 5.50 | 9.30 | 40.86 |
| Water soaked | $T_9$ | 6.96 | 14.30 | 51.75 | 3.34 | 4.63 | 27.86 |
| | Mean | 13.25 | 31.46 | | 5.86 | 11.87 | |
| | CD($p \leq 5$) | 1.93 | 1.68 | | 0.97 | 0.84 | |

The data in Table-4 shows that the maximum seedling quality index of 2.99 and 3.70 during first and second growing season was recorded in the seedlings raised from seeds stratified for 60 days stratification. The seedlings raised from scarified seeds exhibited seedling quality index of 2.09 and 2.14 for the first and second growing seasons respectively and seedlings raised from water soaked seeds produced seedling quality index of 1.37 and 1.46 for the first and second growing seasons respectively. The minimum seedling quality index of 0.97 and 0.98 for the first and second growing seasons was recorded in the seedlings raised from seeds that were not subjected to any treatment (control).

**Table 4:** Effect of pre-sowing treatments on shoot root ratio and seedling quality index of *Ginkgo biloba* seedlings.

| | Treatments | Shoot root ratio | | Seedling quality index ( g mm $cm^{-1}$) | |
|---|---|---|---|---|---|
| | | 2016 | 2017 | 2016 | 2017 |
| Control | $T_1$ | 0.63 | 0.71 | 0.97 | 0.98 |
| Stratification | $T_2$(15 days) | 0.89 | 0.94 | 1.33 | 1.89 |
| | $T_3$(30 days) | 1.24 | 1.37 | 2.44 | 2.66 |
| | $T_4$(45 days) | 1.31 | 1.53 | 2.59 | 2.85 |
| | $T_5$(60 days) | 1.56 | 1.95 | 2.94 | 3.70 |
| | $T_6$(75 days) | 1.06 | 1.52 | 2.52 | 2.76 |
| | $T_7$(90 days) | 1.00 | 1.32 | 2.37 | 2.61 |

| | | | | | |
|---|---|---|---|---|---|
| Scarification | $T_8$ | 0.84 | 1.27 | 2.09 | 2.14 |
| Water soaked | $T_9$ | 0.80 | 1.03 | 1.37 | 1.46 |
| | Mean | 1.03 | 1.29 | 2.07 | 2.34 |
| | CD($p \leq 5$) | N/S | 0.20 | 0.66 | 0.49 |

**Fig 1**

All the germination parameters increased significantly as the stratification period increased. The main reason to explain the effect of chilling on breaking seed dormancy is reflected in enhancing internal GA3 accumulation (Phillips *et al*., 2003) [22]. The embryo of many seeds fails to germinate because oxygen dose not diffuse through the seed coat. At low temperature more oxygen dissolves in water and therefore more oxygen is prepared for embryo (Young and Young, 1992) [27]. Stratification had a significant effect on seed germination of black mulberry (*Morus nigra* L.). There is evidence that stratification might act to lower the rate of enzymatic reactions taking place in the seed and cause differential changes in enzyme concentrations or in enzyme production (Bewley and Black, 1994) [4]. Dormancy break by cold stratification during late autumn to early spring allow seeds to germinate at the beginning of the growing season (Wang *et al*., 2010) [25]. Sofi, (2005) [24] working with *Cedrus deodara* revealed that the stratification treatment beyond 75 days showed declining trend in germination behavior of species. The germination rate of seven types of *Pistacia khinjuk* seeds ranged from 40% to 60% after the seeds were exposed to different treatments, the highest germination rates were obtained with stratified seeds (Kafkas, 1998) [15].

It was evident from the result that growth parameters increased significantly as the stratification period increased from 0 to 60 days. Lakhanpal and Sharma, (1995) [16] reported higher shoot height, root length and stem diameter growth ranging from 11.16cm to 20.6cm, 8.3cm to 14cm and 4.2mm to 5.0mm, respectively for Chilgoza pine seedling. Similar findings have also been reported by Malik, (2007) [18] in *Pinus gerardiana*, Gosling *et al*., (1999) [11] in *Pinus taeda*, Mousavi and Sadat, (2006) [19] in Pyrus species. The results are also in line with Malik *et al.*, (2009) [18] on *Pinus gerardiana*. Fetouh and Hassan, (2014) [10] also reported the enhanced growth of *Magnolia grandiflora* seedlings raised from seeds stratified for a period of 90 days. Pawak, (1993) [23] also reported better seedling growth in Alaska red pine seeds stratified for 60 days under nursery conditions. Various physiological changes occur during the stratification as organic and inorganic constituents accumulate while protein and sugar content increases as the stratification period of seeds increased. This might also be due to conversion of starch into monosaccharide and sucrose, used by embryo for subsequent growth and development (Sofi, 2005; Gautam, 1997) [24, 12].

## References


1. Agrawal PK, Dadlani M. Tecniques in Seed Science and Technology, 2nd Edition., South Asian Publishers, New Delhi. 1995; 109-113.
2. Anonymous. Economic Survey of Jammu and Kashmir state, 2015.
3. Bailey LH. Cultivated evergreens. New York: Macmillen. 1923; 177-178.
4. Bewley JD, Black M. Physiology and Biochemistry of seeds in relation to germination. Berlin: Newyork Springer: Verlag. 1994; 4:181-190.
5. Boralle N, Braquet P, Gottlieb OR. *Ginkgo biloba*: a review of its chemical. IN P. Braquet. (ed). Ginkgolides-chemistry, biology, pharmacology and clinical perspectives. J. R. Prous, Science Publishers. 1988; 1:9-25.
6. Cao FL. Chinese Ginkgo, Jiangsu Science and Tecnology Publishing House, Nanjing. 2003, 460.
7. Cheng J. Maidenhair tree in China. Forestry information. 1993; 13:17-18
8. Dar GH, Khuroo AA, Reddy CS, Malik AH. Impediment to taxonomy and its impact on biodiversity science: an Indian perspective. Proceedings of National Academy of Sciences. 2012; 82:235-240
9. DeFeudis FV. *Ginkgo biloba* extract (EGb 761): From chemistry to clinic, Ullstein Medical, Weisbaden. 1998, 1032-1042.
10. Fetouh MI, Hassan FA. Seed germination criteria and seedling characteristics of *Magnolia grandiflora* L. trees after cold stratification treatments. International Journal Current Microbiology Applied Science. 2014; 3(3):235-241.
11. Gosling PG, Parratt M, Peace A. Identifying the optimum pre-treatment duration, the germination temperature for *Abies nordmaniana* seed and assessing the affect of moisture content and storage temperature on seed longevity. Seed Science and Technology. 1999; 27(3):951-961.
12. Gautam J. Studied on the pre-sowing treatments on germination and seedling development of *Quercus leucotrichophora*. M. Sc Thesis Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P), 1997.
13. Herranz JM, Ferrandis P, Martinez Sanchez JJ. Influence of heat on seed germination of nine woody cistaceae species. International Journal of Wildlife Fire. 1999; 9(3):173-182.
14. Jacobs BP, Browner WS. *Ginkgo biloba*: a living fossil. American journal of Medicine. 2000; 108(4):341-342.
15. Kafkas S, Kaska N. The effect of scarification, stratification and GA3 treatments on the germination of seeds and seedling growth in selected *Pinus khinjuk* types. Acta Horticulturae. 1998; 470:545-459.
16. Lakhanpal TL, Kumar S. Regeneration of Cold Desert Pine of N. W. Himalayas (India) – A Preliminary Study. In: Roundy *et al. (eds.)* Proceedings: wild land shrub and arid land restoration symposium, Las Vegas, NV. 1993, 1995, 102-106.
17. Masood E. Medicinal plants threatened by over use. Nature. 1997; 66:570.

18. Malik AR, Shamet GS, Majid A. Germination and seedling growth of *Pinus gerardians* in nursery, Effect of stratification period and temperature. Indian Journal of Forestry. 2009; 32(2):221-225.
19. Mousavi ZA, Saadat YA. Breaking of dormancy and germination of wild Pear (*Pyrus species*) seeds. Iranian Journal of Rangelands and Forest plant Breeding and Genetic Research. 2006; 14(2):92-94.
20. Narbona E, Arista M, Ortiz PL. Seed Germination of *Arbutus unedo* L. (Ericaceae). Acta Botanica Malacitana. 2003; 28:73-78.
21. Ponder HG, Shumack RL, Gillen CH. Liners: the first step in shade tree production. American nurseryman. 1981; 153(11):10-64.
22. Phillips N, Drost D, Varga W. Chemical treatments enhanced seed germination in *Perideridia gairdneri*. Acta Horticulturae. 2003; 618:477-482.
23. Pawak WH. Germination of Alaska Cedar seed. Tree Planter's Notes. 1993; 44(1):21-24.
24. Sofi PA. Standardization of nursery technology of *Cedrus deodara*. Ph. D. Thesis, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni – Solan (H. P), India. 2005; 176.
25. Wang JH, Baskin CC, Chen W, Du GZ. Variation in seed germination between populations of five sub-alpine woody species from eastern Qinghai-Tibet Plateau following dry storage at low temperature. Ecological Research. 2010; 25(1):195-203.
26. Willan RL. A guide to forest seed handling with special reference to the tropics. For. Rome: FAO. 1985, 2.
27. Young JA, Young CG. Seeds of Woody plants in North America, Dioscorides Press, Portland, Oregon. 1992; 310-312.
28. Wang BSP, Berjak P. Beneficial Effects of Moist Chilling on the seeds of Black Spruce (*Picea mariana* [Mill.] B. S. P.), Annals of Botany. 2000; 86(1):29-36.