

# Seed Morphology and Germination Studies on *Carpinus viminea* for Conservation in North-Western Himalaya

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**ABSTRACT:** *Carpinus viminea* (Himalayan Hornbeam) is one of the rare and multipurpose tree species of the Indian Himalayan Region. Over-exploitation for fuel, fodder and house building, habitat degradation and poor regeneration of species may lead to the extirpation of species. Therefore, seed morphology, seed viability and seed germination were investigated. Seeds were collected from Kanawar (2244m), Kandhi (1952m) and Khokhan (1472m) populations in Kullu district of Himachal Pradesh. They were dried for a week at room temperature and stored at 4°C in refrigerator. Tetrazolium test was used for the seed viability. The stored seeds were treated with gibberellic acid ( $GA_3$  15, 25 and 35 $\mu$ M), indole acetic acid (IAA 15, 25 and 35 $\mu$ M) and potassium nitrate ( $KNO_3$  130, 150 and 170mM). Maximum seed viability (80.0%, each) was recorded in Kanawar and Kandhi populations. 1000 seed weight increases with the increase in altitude. Maximum seed length (4.19 $\pm$ 0.05mm), 1000 seeds weight (9.2gm), seed width (3.10 $\pm$ 0.02mm) and seed thickness (1.92 $\pm$ 0.02) were recorded in Kanawar population. Therefore, seeds collected from Kanawar population were selected for germination. In control condition, seed germination and Mean Germination Time (MGT) was poor. Treatments applied to the seeds improved germination % significantly ( $F = 14.793$ ,  $p < 0.001$ ) over control. Highest germination percentage (76.67%) was achieved in  $GA_3$  35 $\mu$ M and  $KNO_3$  130 mM. Minimum time for first seed germination (11 days) was observed in  $KNO_3$  170 mM and minimum MGT (13.31 days) was observed in  $KNO_3$  150 mM. Therefore, regular monitoring and complete protection of habitats for *in-situ* conservation are suggested. Seed germination protocol developed can be used for mass multiplication of species and establishment and maintenance of seedlings in suitable habitat (*in-situ* condition) need to be encouraged with the help of local inhabitants and Forest Department.

**Keywords:** *Carpinus viminea*, Multipurpose, Rare, Seed Morphology, Seed Germination, Viability, North Western Himalaya, Conservation

There is now widespread acceptance among foresters, governmental agencies, and environmental groups that forest land cover is shrinking and disappearing at an alarming rate due to various natural and anthropogenic activities. Forest cover of Indian Himalaya Region (IHR) is also reducing at an alarming rate [1]. The IHR covers an area of 59 million ha of which 22 million ha is degraded [2]. The mountainous belt of the IHR present unique environment and support many multipurpose tree species (MPTs). Amongst the MPTs, *Carpinus viminea* Wall. ex Lindl. (Himalayan Hornbeam) is a notable species, and is native to the Himalaya. It is distributed across the Himalaya to Korea and Vietnam between 1200 - 2600 m amsl. In the IHR, the species has been reported from Central Himalaya [3-11], North-Western Himalaya [12-14], North-Western and Western Himalayan [15] and North-Eastern Himalaya [16-18]. It usually occurs in isolated patches mainly on the low

canopy areas of oak forests, rarely mixed with evergreen and deciduous tree species. Wood is moderately hard and used mainly for making small timber, furniture and articles of sports as well as weaving shuttle, musical instruments, agriculture implements, leaves used as fodder, seeds and buds are eaten by birds [6, 12, 19-24] and also used for medicinal practices [25]. In general, a large number of studies have been carried out on ethno botany / resource utilization pattern of the species and mentioned in the research papers [12, 25-29], Ph. D. thesis [6, 13, 14, 30], D.Sc. thesis [15] and floras [22, 31, 32]. However, in particular, in *Carpinus viminea*, the studies are restricted to ethno botany / resource use pattern, seed maturity indices, and seedling character. But, none of the studies have investigated the species for seed morphology, seed viability, development of conventional propagation (seed germination) protocols, and suggest species specific management options for

conservation. Therefore, the present study was conducted on the above parameters for the conservation of *C. viminea* and sustainable development of the Himalayan forests.

## MATERIALS AND METHODS

Present study was conducted in the Kullu District of Himachal Pradesh North-Western Himalaya. Seeds of *C. viminea* were collected from three different populations (i.e., Kanawar (2244 m), Kandhi (1940 m) and Khokhan (1472 m). All the sites were located between 31.83°N - 31.99°N latitudes and 77.08°E - 77.32°E longitudes in moist slope habitat and north aspect with slope ranging from 10 - 60° (Table 1 & Figure 1).

**Table 1.** Physical characteristics of sites supporting populations of *C. viminea*

Populations	Habitat	Latitude	Longitude	Aspect	Slope	Altitude
Kanawar	Moist slope	31.99°N	77.32°E	North	10°	2244 m
Kandhi	Moist slope	31.83°N	77.09°E	North	60°	1952 m
Khokhan	Moist slope	31.88°N	77.08°E </tr			



**Figure 1.** Map showing the studied populations of *C. viminea*

### Seed Collection

Seeds were collected in late October and early November from three different selected populations. The best seed maturation period for Himalayan Hornbeam is late October and early November, thus, seeds were

collected during this period. Collected seeds were dried for a week at room temperature. The extra materials from seeds were removed and predated/damaged seeds were separated. Healthy seeds were stored at 4°C in the refrigerator.

### Seed Morphology

Length, width and thickness of 30 seeds from each population were measured by Electronic Vernier Caliper (Model CD-8" CS, Mitutoyo, Japan). Total 800 seeds (eight replicates of 100 seeds) were collected randomly from each population and then their 100 seed weight were measured based on ISTA rules with a micro-electronic balance (ANAMED, model MA7301A).

### Tz (Triphenyl tetrazolium chloride) Test

Thirty seeds of each population were imbibed in distilled water for 12 h, followed by dissection of seeds transversely 1/4 to 1/3 at the end away from embryo into equal halves [33]. Half of the dissected embryos were then placed in petri dishes containing 0.5% solution of Tz. The petri dishes were then wrapped in aluminium foil and incubated for 4 h, after which the embryos were washed and observed for red colouration. The coloured embryos were counted as viable. Percentage viability from three replicates were recorded [34].

### Germination Test

In February 2015, air dried seeds were surface sterilized by dipping in 0.1% aqueous solution of Mercuric Chloride ( $\text{HgCl}_2$ ) to discourage fungal infection, washed thoroughly with double distilled water (DDW) in all cases [34] and dipped in various pre-treatment solutions (24 h,  $25 \pm 2^\circ\text{C}$ , dark). These include gibberellic acid ( $\text{GA}_3$ ; 15, 25 and 35  $\mu\text{M}$ ); indole acetic acid (IAA; 15, 25 and 35  $\mu\text{M}$ ) and potassium nitrate ( $\text{KNO}_3$ ; 130, 150 and 170 mM). Control was maintained using DDW. To achieve high germination of seeds, pre-sowing chemical treatments such as  $\text{GA}_3$ , IAA and  $\text{KNO}_3$  were used. Treated seeds were washed two or three times with DDW and placed in petri dishes lined with filter paper (four replicates/treatment, 50 seeds/replicate) [35] in completely randomized design under laboratory conditions (average temperature:  $25 \pm 0.5^\circ\text{C}$ ) and monitored daily at 10:00 AM. The filter papers were moistened daily using DDW. Seeds were considered germinated upon the redical emergence [36] and the average was expressed in percentage.

## Data Analysis

The data was analyzed with the help of MS-Excel 2007 and mean values of treatments with standard deviation were calculated. ANOVA was used to interpret the variation and to identify the best treatment. Statistical software SYSTAT 13 (SYSTAT Software, Inc.2013) was used for the analysis of variance among different treatments and Mean Germination Time (MGT). MGT was calculated by following Nichols and Heydecker [37] and Viability Adjusted Germination (VAG) was calculated following Roche *et al.* [38].

## RESULTS AND DISCUSSION

### Seed Morphology

Among the seeds of the three naturally growing populations, weight of 1000 seeds was highest in Kanawar population ( $9.2 \pm 0.02$  gm), followed by Kandhi ( $6.1 \pm 0.01$  gm) and Khokhan ( $5.8 \pm 0.01$  gm) populations. Seed length was highest in Kanawar population ( $4.19 \pm 0.05$  mm), followed by Khokhan ( $4.16 \pm 0.03$  mm) and Kandhi ( $3.69 \pm 0.03$  mm) populations. Seed width was highest in Kanawar population ( $3.10 \pm 0.02$  mm), followed by Khokhan ( $2.95 \pm 0.03$  mm) and Kandhi ( $2.55 \pm 0.02$  mm) populations. Seed thickness was highest in Kanawar population ( $1.92 \pm 0.02$  mm), followed by Khokhan ( $1.86 \pm 0.02$  mm) and Kandhi ( $1.68 \pm 0.02$  mm) populations. There was significant difference among the weight of 1000 seeds ( $F = 1443.57$ ;  $p < 0.001$ ), seed length ( $F = 62.81$ ;  $p < 0.001$ ), seed width ( $F=145.70$ ;  $p < 0.001$ ) and seed thickness ( $F = 42.72$ ;  $p < 0.001$ ) of different populations (Table 2).

### Germination Test

Maximum seed viability was recorded in Kanawar and Kandhi populations (80.0%, each) followed by Khokhan

population (60%). Mean germination percentage for control R (room temperature) and control BOD (Biological Oxygen Demand Incubator) was observed 3.33% and 50%, respectively. First seed germination was observed in 14 days under control BOD and 26 days in control R. Mean germination time (days) for control and control BOD was observed in 22.33 days and 17.92 days, respectively. One Way Analysis of Variance (ANOVA) showed difference in germination ( $F = 14.80$ ;  $p < 0.001$ ), and was significant. However, difference in mean germination time was not significant. Among the concentration of growth regulators, highest germination percentage (76.67%) was achieved in  $GA_3$  35 $\mu$ M and  $KNO_3$  130mM, respectively. Minimum time for first seed germination (11 days) was observed in  $KNO_3$  170mM. Minimum mean germination time (13.31 days) was observed in  $KNO_3$  150mM (Table 3).

### Gibberellic acid ( $GA_3$ ) treatment

In  $GA_3$  (15 $\mu$ M), first seed germination was observed in 12 days, followed by 12.67 days in 35 $\mu$ M  $GA_3$ ; 13.67 days in 25 $\mu$ M  $GA_3$ . Mean germination time (days) for 35 $\mu$ M  $GA_3$  was observed in 15.52 days, followed by 15.58 days in 15 $\mu$ M  $GA_3$  and 18.36 days in 25 $\mu$ M  $GA_3$ . Mean germination percentage for 15 $\mu$ M  $GA_3$  and 25 $\mu$ M  $GA_3$  70%, followed by 76.67 % in 35 $\mu$ M  $GA_3$  was observed (Figure 2).

### Indole acetic acid (IAA) treatment

First seed germination was observed in 12 days for IAA 15 $\mu$ M, followed by 12.33 days in IAA 35 $\mu$ M and 14.67 days in IAA 25 $\mu$ M. Lowest mean germination time (days) for 25 $\mu$ M IAA was 16.08 days, followed by 16.86 days in 15 $\mu$ M IAA and 17.04 days in 35 $\mu$ M IAA. Mean germination percentage for IAA 25 $\mu$ M was observed 55%, followed by 68.33% in IAA 35 $\mu$ M and 70% in IAA 15 $\mu$ M (Figure 2).

**Table 2.** Morphological characters of seeds in different populations of *Carpinus viminea*

Population		Weight of 1000 seed	Seed Length (mm)	Seed Width (mm)	Thickness (mm)
Kanawar	Mean $\pm$ SE	9.2 $\pm$ 0.02 gm	4.19 $\pm$ 0.05	3.10 $\pm$ 0.02	1.92 $\pm$ 0.02
	Range	0.88-0.94	3.73-4.72	2.87-3.34	1.74-2.15
Kandhi	Mean $\pm$ SE	6.1 $\pm$ 0.01 gm	3.69 $\pm$ 0.03	2.55 $\pm$ 0.02	1.68 $\pm$ 0.02
	Range	0.57-0.60	3.28-3.93	2.33-2.79	1.44-1.89
Khokhan	Mean $\pm$ SE	5.8 $\pm$ 0.01 gm	4.16 $\pm$ 0.03	2.95 $\pm$ 0.03	1.86 $\pm$ 0.02
	Range	0.60-0.62	3.86-4.47	2.71-3.23	1.64-2.12
F-value		1443.57*	62.81*	145.70*	42.72*
p-value		0.000	0.000	0.000	0.000

\*Significant at  $p < 0.05$  level

**Table 3.** Effects of pre-sowing chemical treatments on germination of *C. viminea* seeds under laboratory condition (n = 50 seeds/replicate)

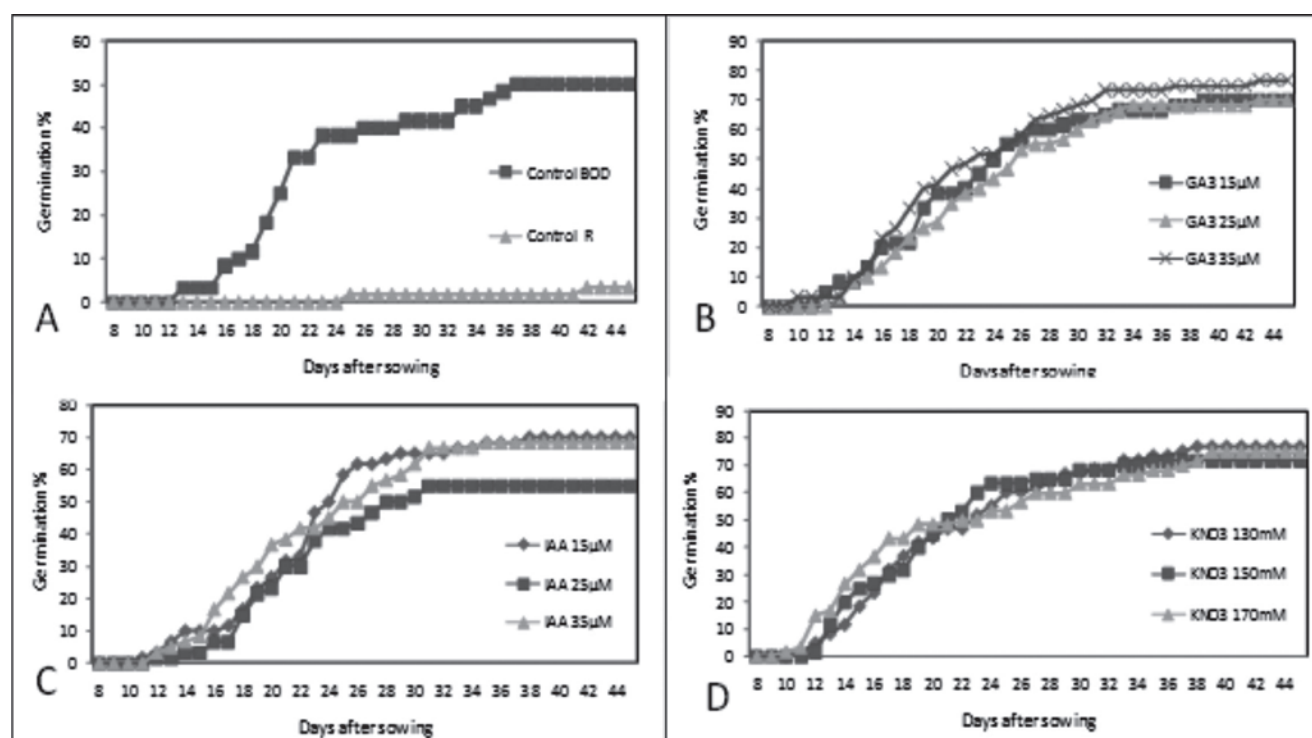
Treatment	Seed germination (%) $\pm$ SE	Viability adjusted germination (%) $\pm$ SE	First germination time $\pm$ SE (days)	Mean germination time $\pm$ SE
Control R	3.33 $\pm$ 1.67	4.14 $\pm$ 2.09	22.33 $\pm$ 12.21	22.33 $\pm$ 12.21
Control BOD	50.00 $\pm$ 7.47	62.50 $\pm$ 9.56	14.00 $\pm$ 1.00	17.92 $\pm$ 1.34
GA <sub>3</sub> 15 $\mu$ M	70.00 $\pm$ 5.78	87.50 $\pm$ 7.23	12.00 $\pm$ 0.00	15.58 $\pm$ 2.17
GA <sub>3</sub> 25 $\mu$ M	70.00 $\pm$ 0.00	87.50 $\pm$ 0.00	13.67 $\pm$ 0.33	18.36 $\pm$ 0.87
GA <sub>3</sub> 35 $\mu$ M	76.67 $\pm$ 6.67	95.83 $\pm$ 8.34	12.67 $\pm$ 1.33	15.53 $\pm$ 2.06
IAA 15 $\mu$ M	70.00 $\pm$ 7.65	87.50 $\pm$ 9.56	12.00 $\pm$ 0.58	16.86 $\pm$ 1.86
IAA 25 $\mu$ M	55.00 $\pm$ 5.01	68.75 $\pm$ 6.25	14.67 $\pm$ 1.77	16.08 $\pm$ 2.23
IAA 35 $\mu$ M	68.33 $\pm$ 7.27	85.42 $\pm$ 9.09	12.33 $\pm$ 0.33	17.04 $\pm$ 1.06
KNO <sub>3</sub> 130mM	76.67 $\pm$ 4.41	95.83 $\pm$ 5.52	12.00 $\pm$ 0.58	16.19 $\pm$ 2.09
KNO <sub>3</sub> 150mM	71.67 $\pm$ 4.41	89.58 $\pm$ 5.52	12.67 $\pm$ 0.33	13.31 $\pm$ 1.97
KNO <sub>3</sub> 170mM	75.00 $\pm$ 5.01	93.75 $\pm$ 6.26	11.00 $\pm$ 0.58	16.34 $\pm$ 1.59
F-value	14.793*	14.793*	NS	NS

\*Significant at p < 0.05 level; NS= Non-significant

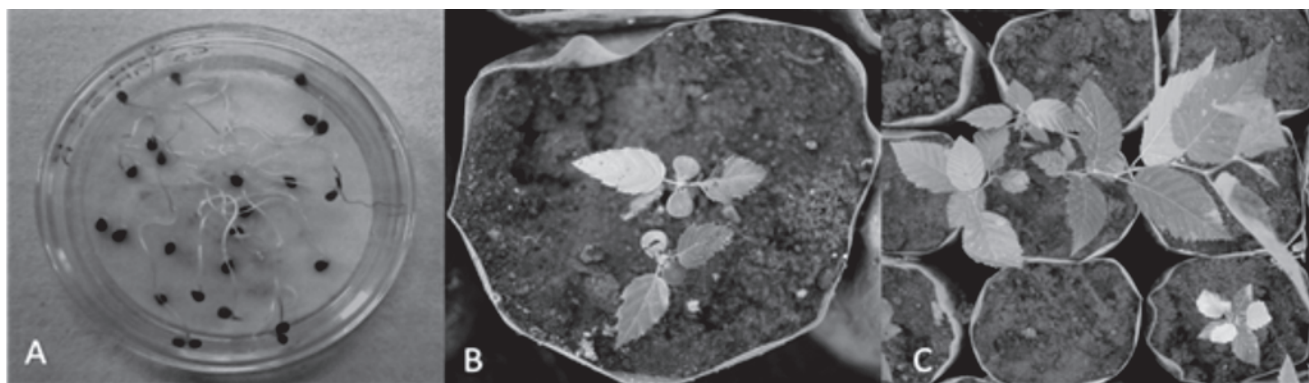
### Potassium nitrate (KNO<sub>3</sub>) treatment

First seed germination for control BOD was observed in 14 days. In 170mM KNO<sub>3</sub>, it was 11 days, followed by 12 days in 130mM KNO<sub>3</sub> and 12.67 days in 150mM KNO<sub>3</sub>. Mean germination time (days) for control BOD was 17.92 days. In 150mM KNO<sub>3</sub>, MGT was 13.31 days, followed

by 16.18 days in 130mM KNO<sub>3</sub> and 16.34 days in 170 $\mu$ M KNO<sub>3</sub>. Mean germination percentage for control BOD was 50%. In 150 $\mu$ M KNO<sub>3</sub>, it was 71.67%, followed by 75% in 170 $\mu$ M KNO<sub>3</sub> and 76.67% in 130 $\mu$ M KNO<sub>3</sub>. Germinated seeds were transferred to shade house and poly house (Figure 2 & Plate 1).



**Figure 2.** Time-course changes in germination percentage of *C. viminea* seeds pretreated with different growth regulators: A) water soak and the control; B) gibberellic acid; C) indole acetic acid and; D) potassium nitrate



**Plate 1.** Germination of *Carpinus viminea*: A in laboratory, and B & C in polyhouse

Nearly, all cultures from ancient times to the present day have used plant resources as medicine, food/wild edible, fodder, fuel, timber, religious, dye and various other purposes [39]. Indian Himalaya Region supports many multiple purpose tree species. Among these species, *Carpinus viminea* is one of the rare species and used as fodder, fuel, timber, medicinal, etc. [13, 14]. This species usually occurs in isolated patches mainly on the low canopy areas of oak forests as major associated species. The natural regeneration of the species has been affected badly and population of the species is depleting fast, due to various anthropogenic and natural activities. Therefore, the present study was conducted on seed morphology, seed viability, development of conventional propagation (seed germination) protocol for mass multiplication, and to suggest suitable management options for the conservation of the species.

To achieve high germination in plants, use of pre-sowing chemical treatments such as  $GA_3$  [40, 41], IAA [42] and  $KNO_3$  [43, 44] are recommended. Seeds of *Carpinus viminea* possess high viability. Most of the treatments used in this study improved germination and MGT. Treatment of seeds with  $35\mu M GA_3$  and  $130mM KNO_3$  increased mean germination percentage to 76.67% as compared to 50% in control BOD condition and 3.33% in control R. High germination percentage and lower MGT was also observed in  $150mM KNO_3$  (71.67% with 13.31 days MGT) and  $150mM KNO_3$  (75% with 16.64 days MGT). From the above results, it can be concluded that growth regulators and chemical compounds play significant role in the germination of seeds of *Carpinus viminea* as compared to control condition.

Seeds that were collected from Kanawar population were heavier (9.2 gm) than Kandhi (6.1 gm) and Khokhan

(5.8 gm) populations. There was significant difference among the 100 seed weight, seed length, seed width and seed thickness of different populations. Seed width, length and thickness were also higher in Kanawar population as compare to other populations. These results showed that 100 seed weight and seed fullness increases as altitude increased. Also, the habitat preference of the species play important role in the development of seeds, seedlings, saplings and trees of the species. This result is similar to the study of Bhatt and Ram [23]. In spite of high viability, species is not able to germinate and proliferate in the natural habitats. This may be due to over exploitation, heavy grazing and competition between associate species. Therefore, regular monitoring and complete protection of habitats for *in-situ* conservation are suggested. Seed germination protocol developed can be used for mass multiplication of species and establishment and maintenance of seedlings in suitable habitat (*in-situ* condition) need to be encouraged with the help of local inhabitants and Forest Department.

## CONCLUSION

The use of multipurpose tree species is enormously increasing in Indian Himalayan Region. This has put a high pressure on such ecologically and economically important biodiversity elements. The various *ex-situ* as well as *in-situ* conservation efforts need to be geared to combat the existing challenges. The present study has developed seed germination protocol for *C. viminea*. The study concludes that gibberellic acid ( $35\mu M GA_3$ ) and potassium nitrate ( $130mM KNO_3$ ) play significant role in seed germination of Hornbeam. Therefore, there is a need to the mass multiplication of the species in the nurseries and their establishment in the natural habitat with participation of local inhabitants and Forest

Department. Also, there is a need to monitor the performance of the seedlings and protect habitats for ensuring the *in-situ* conservation.

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