



**RESEARCH ARTICLE**

**SEED MATURITY INDICES IN CARPINUS VIMINEA (HIMALAYAN HORNBEAM)  
ALONG ALTITUDINAL GRADIENTS IN RELATION TO CLIMATE CHANGE**

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**ABSTRACT**

Seed maturity and other phenological studies acquire a new importance because its relation to climate change and can be used to predict the adaptiveness of a species. We have investigated maturity period of a rare distributed, under canopy tree *Carpinus viminea* (Himalayan Hornbeam). The main objective of the study is to find out seed maturity period along the environmental gradient in relation to change in climatic conditions (especially the temperature and rainfall) for its better regeneration, conservation and multiplication. The study indicated that seed moisture negatively relation with germination ( $r^2=0.43$ , 29df,  $p<0.001$ ) while positively with seed fresh weight ( $r^2=0.34$ , 29df,  $p=0.001$ ). Elevation showed positive relation for both seed germination ( $r^2=0.38$ , 29df,  $p=0.002$ ) and filled seed production ( $r^2=0.40$ , 29df,  $p<0.001$ ). Seed maturity period was found in the first week of November. The elevations 2100 to 2300m are the potential for its seed collection and regeneration programs. Early flowering due to temperature rise and high rainfall during flower maturation may be responsible for low pollination success and could be the reason for low sound seed percentage consequently the germination in year 2<sup>nd</sup>. Such erratic climatic irregularities will likely to be more pronounced in coming future due to climate change. Under such conditions existence of the species below 2000m in will be in jeopardy.

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**INTRODUCTION**

With evidence of global warming and biological response to that warming accumulating rapidly (Parmesan, 2006; IPCC, 2007 a; b) and considerable attention has turned to predicting the fate of trees and forests. Plant phenology is one of the most widely used trait and has acquired a new importance because its relation to climate changes (Chapman *et al*, 2005). Phenology is sensitive to environmental cues such as temperature, moisture and photoperiod (Rathacke and Lacey, 1985). Seed maturation after flowering, pollination, fertilization and seed development is an integral part of phenology and in fact, it decides future development of forest. As for its reproductive function, seed maturation is one of the most important parts of the life cycle in forest trees. Variation in maturation time is the most robust way to study the effect of anticipated climate change on forest tree species. Change in maturity period can change germination and growth rate; hence change the structure and composition of a stand in due time course. Thus, seed maturation study is important for conservation, development, growth, management (Bhatt and Ram, 2009) of a species and its adaptation in changing climatic conditions. Further, the time of maturation is also important for the collection, storage and testing of seeds for its better regeneration. In the context of the sensitivity analysis of climate change, lesser known under-canopy tree species

(LKTS) species are to be considered important. We believe that the impact of climate change is more on LKTS owing to their restricted distribution coupled with shade loving habit. Warming on such species can influence offspring germination, survival, finally regeneration and stand structure. Unfortunately, these species receives little attention till recent years by the researchers and policy makers despite their diversified ecological and socioeconomic uses. Therefore, it is imperative to make a database of these species to understand the impact of climate change on maturation and its effect on regeneration.

*Carpinus viminea* Lind. (Himalayan Hornbeam, family Corylaceae), an under canopy, lesser known tree species was selected for seed maturity indices in its natural distribution range. It is a medium sized, tree distributed between 1700-2400 m asl in Uttarakhand Himalaya. It occurs either in isolated patches of about 1-2 ha or mixed with other evergreen and deciduous tree species, mainly as an understory in oak dominated moist Himalayan temperate forests under early successional conditions (Bhatt and Ram, 2009). The male catkins (staminate aments) and female (pistillate aments) appear along with the new leaves in spring on the same tree. Pollination occurs during March- April. Fruits are ovoid ribbed; single seeded nutlets each born at the base of a distinct three lobed involucre (bract) in clusters of 12-25 at the top of

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young shoots (Bhatt and Ram, 2005). The wood is moderately hard and is mainly used for making musical instruments, bobbins and shuttles in textile industry, tool handles and sometimes also for making small timber. Leaves are used as fodder, seeds and buds are eaten by a number of birds specially the parakeets (Bhatt, 2005). The objectives of the present study are (i) to ascertain appropriate seed harvest period through maturation indices (ii) to find out variation in seed maturity period along the environmental gradient in relation to temperature and rainfall (climatic variables) (iii) to make a database for long term climate change study for its better regeneration, conservation and multiplication.

## MATERIALS AND METHODS

### Study area

The study area is located between 29° 23' and 29° 26' N and 79° 25' and 79° 28' E in the Himalayan zone of Uttarakhand (Table 1). *Quercus leucotrichophora* A. Camus is the dominant forest forming tree species at low elevation, *Q. floribunda* Lindley - mixed forests at middle elevations while *Q. lanata* Smith - mixed oak forests dominated at high elevations. The study area experienced with anthropogenic disturbances in the form of forest fire and deforestation, which accelerates soil erosion and nutrient loss from these valuable forest ecosystems (Bhatt and Ram, 2009). Monsoon pattern of climate is prevalent with average annual rainfall (average of three years) varied from 1789 mm at 1800 m and 1059.3 mm at 2300 m elevation, of which nearly two-third occur during monsoon period (mid June – mid September). Mean monthly temperature ranged between 5.4° C (January) and 20.8° C (May) while mean daily temperature ranged between -5.0° C and 33.0° C. Both rainfall and temperature decreased as the elevation increased. Mean annual temperature decreased 0.5 °C with increase of 100 m elevation whereas no clear trend was observed in annual rainfall. 30 cm snowfall occurs from December to February above 2000 and 2300 m elevations.

Seeds were collected when they were sufficiently developed. Seed collection in both the years started from 16<sup>th</sup> October (32 week after anthesis) and continues till >85% seed fall completed, at weekly interval. Seeds were collected directly from the marked trees by an expert tree climber brought to the laboratory and manually separated from involucre. Collected seeds of a date from all the trees of an elevation was mixed thoroughly to make a composite seed lot. Four replicates from each lot were used to determine seed size (10 seeds each) and fresh weight of 100 seeds. Seed size was the product of length and width, measured with the help of digital vernier and weight by digital balance. Percent seed moisture was determine on fresh weight basis using four replicates of 1 gm each oven dried at 103±1° C for 16 ±1 hr and reweighed (ISTA, 1993). Four replicates each of 50 seeds of a collection from each elevation lot subjected to cutting test to categorize as filled (complete embryo and endosperm) and empty (without embryo and undeveloped embryo) seeds.

Germination experiment was made at Kumaun University Campus, Nainital situated at an elevation of 2000m asl. Four replicates of 100 seeds from each seed lot, at each collection were used for germination test. Germination test was conducted using 6'' diameter glass petridishes in a dual door seed germinator at 20.0±1.0° C for 8 hr on top of the germination paper. Seed fall in the species occurs during November and the seeds remain in the soil over the winter at low temperature and germinate during the next summer when the mean monthly temperature is approximately 18-20°C, thus, seed germination temperature was considered 20° C (Bhatt and Ram, 2005). Germination was counted daily and seeds were considered germinated when visible protrusion of radical reached about 2 mm in length. Germination was monitored for six weeks with distilled water being added as required. The results were expressed following Pandit *et al* (2002), Pandit and Ram (2004) and Bhatt and Ram (2007; 2009).

**Table 1** Site and tree characteristics of *C. viminea* at different elevations in the Himalayas.

Elevation (m)	Aspect	Latitude N	Longitude E	Density (ind ha <sup>-1</sup> )	Height (m)	dbh (cm)	Dominant tree species
1800	NE	29°24'05.1''	79°27'54.1''	26± 5	21.6± 0.5	35.2± 3.5	<i>Quercus leucotrichophora</i>
1900	NE	29°23'58.1''	79°27'58.8''	24± 5	20.0± 1.2	33.9± 2.1	<i>Q. floribunda</i>
2000	N	29°25'48.9''	79°25'58.5''	28± 7	17.2± 1.1	35.0± 1.8	<i>Q. floribunda</i>
2100	N	29°23'11.5''	79°26'52.9''	30± 4	18.4± 0.7	40.1± 1.1	<i>Q. floribunda</i>
2200	NW	29°25'06.3''	79°26'10.3''	48± 7	19.2± 1.0	31.6± 2.7	<i>Q. lanata</i>
2300	NE	29°25'15.1''	79°26'20.6''	62± 7	17.2± 1.4	35.0± 3.1	<i>Q. lanata</i>

### Data collection Germination

After a thorough reconnaissance of the area six elevations in natural distribution range of the species were selected for the study (Table 1). At each elevation, five average, normal, healthy and diseased free trees were selected and marked at a distance of 50-100 m to minimize cross pollination. With increase in elevation the slope also increased and soil moisture decreased. Mean tree density in the stand ranged between 16± 2.0 and 62± 7.0 individuals ha<sup>-1</sup> across the elevations. Mean height of the selected trees ranged between 17.2± 1.4 and 21.6± 0.5m while diameter between 31.6± 2.7 and 41.1± 1.1cm (Table 1). ANOVA indicated that there were no significant variations in tree parameter along the elevational gradient.

### Data Analysis

Data analysis was carried out using SPSS programme version 12 (SPSS, 2003). The data were analyzed for analysis of variance (ANOVA) to test the difference in seed parameter among the elevations and date of collection. Effect and interaction of elevations and date of collection on seed size, weight, moisture content, filled seeds, germination percentage, was determined taking four replicates using factorial model of GLM multivariate.

Experimental design for all parameters, except germination was randomized block whereas for germination it was completely randomized block. Elevation comprises the main plots with

years and collection dates nested within elevation as subplots. Paired t-test was used to observe the difference more precisely between the year seed parameters and least significant difference (LSD) among the elevations and collection dates. Linear correlation was also made between seed weight vs germination, seed moisture vs germination, filled seed percentage vs elevation, germination percentage vs elevation. The t-test was also used to observe the variation in temperature along the elevational gradient as well as between the years and months.

## RESULTS

### Temperature and rainfall

Yearly variation in temperature between the elevations was not observed except 1800m which was cooler ( $t_{<0.001}=5.84$ , 11df) in 1<sup>st</sup> year. However, temperature of the same months between the years varied significantly for January ( $t_{<0.05}=2.09$ , 5df) and August ( $t_{<0.05}=2.37$ , 5df) which were hotter while October ( $t_{<0.05}=2.26$ , 5df) was cooler in the year 2<sup>nd</sup>. Variation in temperature between the months of the same year was found for September and October of year 2<sup>nd</sup>. It decreased significantly ( $t_{<0.05}=6.2$ , 5df) in the year 1<sup>st</sup> but not ( $t_{<0.05}=0.8$ , 5df) in the 2<sup>nd</sup> year. Total rainfall varied ( $t_{<0.05}=1.84$ , 11df) significantly between the years. It was 1261.3mm (average of all elevations) in the year 1<sup>st</sup> while was 1821.6mm in the year 2<sup>nd</sup>.

### Seed characteristics

Various seed characteristics in different elevations are given in Fig. 1. ANOVA indicated that seed size ( $f=6.9$ , 5df), seed fresh weight ( $f=78.2$ , 5df), seed moisture ( $f=173.8$ , 5df) and seed germination percentage ( $f=216.3$ , 5df) were varied significantly ( $p<0.001$ ) among the elevations. Seeds of 2300 m elevation were largest in size followed by 2000 and 2200 m whereas seeds of 1900m had smallest size. LSD indicated that the seeds of 1800m elevation were significantly varied in size from the 2000 and 2300 m and seeds of 1900m varied from 2000, 2200 and 2300 m elevations.

Fresh weight of seeds increased with increase in elevation. LSD indicated that the fresh seed weight varied significantly among the studied elevations except for 1800 m and 2300 m. Seed moisture showed decreasing trend with increase in elevation. LSD showed that seed of 1900 m, 2100 m and 2300 m elevations significantly varied in moisture percentage. Germination percentage of seed increased with increase in elevation. It increased from 20.5 % at 1800 m altitudes to 47.8 % at 2300 m elevation.

LSD showed that the germination percentage varied among the elevations except between 1900 and 2000 m and emptiness in seeds decreased with increase in elevation. LSD showed that seed emptiness varied among the studied elevations except between 1900 and 2000 m and between 2100 and 2200 m elevations. Generally, with increase in elevation seed moisture and emptiness percentage decreased and the seed germination rate and germination percentage increased.

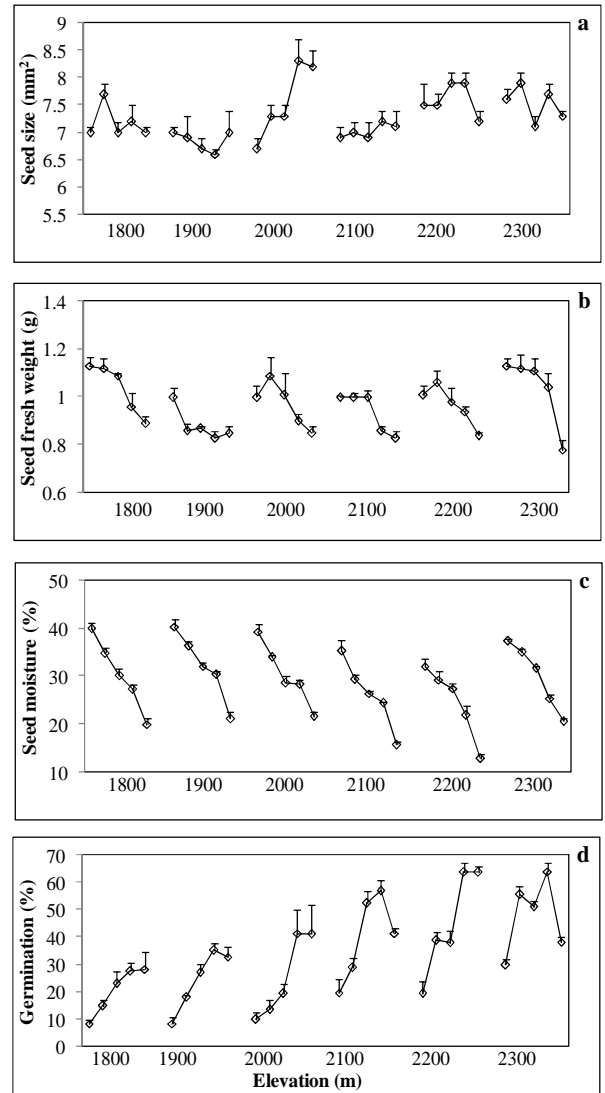


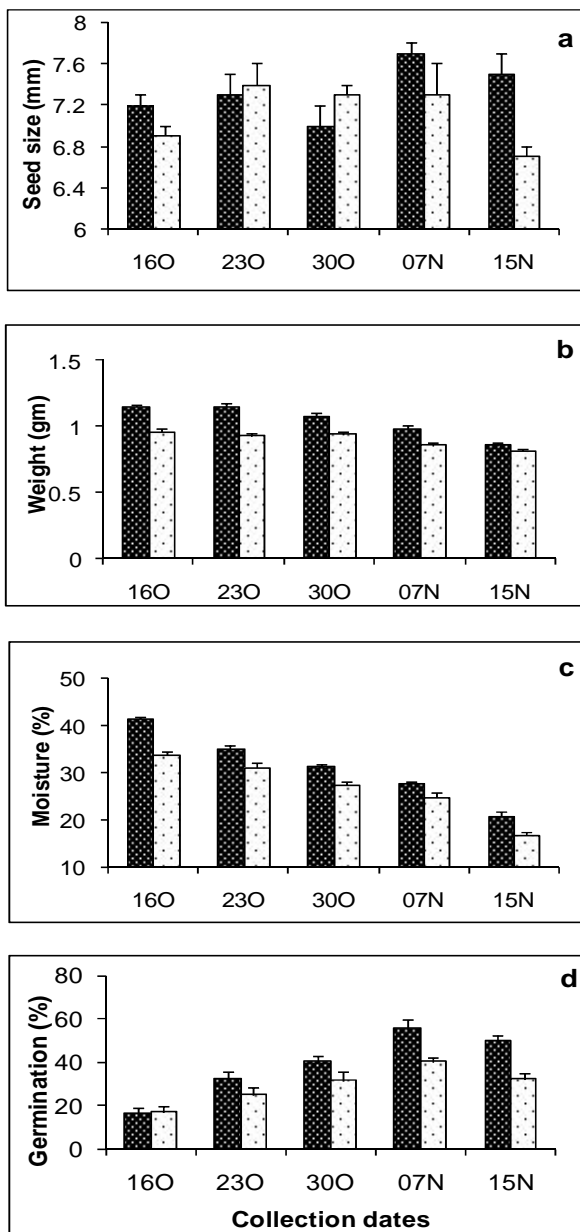
Figure 1 Seed size, seed fresh weight, moisture and germination percentage of *C. viminea* at different elevations. Each circle at each elevation represents a collection date. Error bars indicate  $\pm$  Se.

### Variation between the years

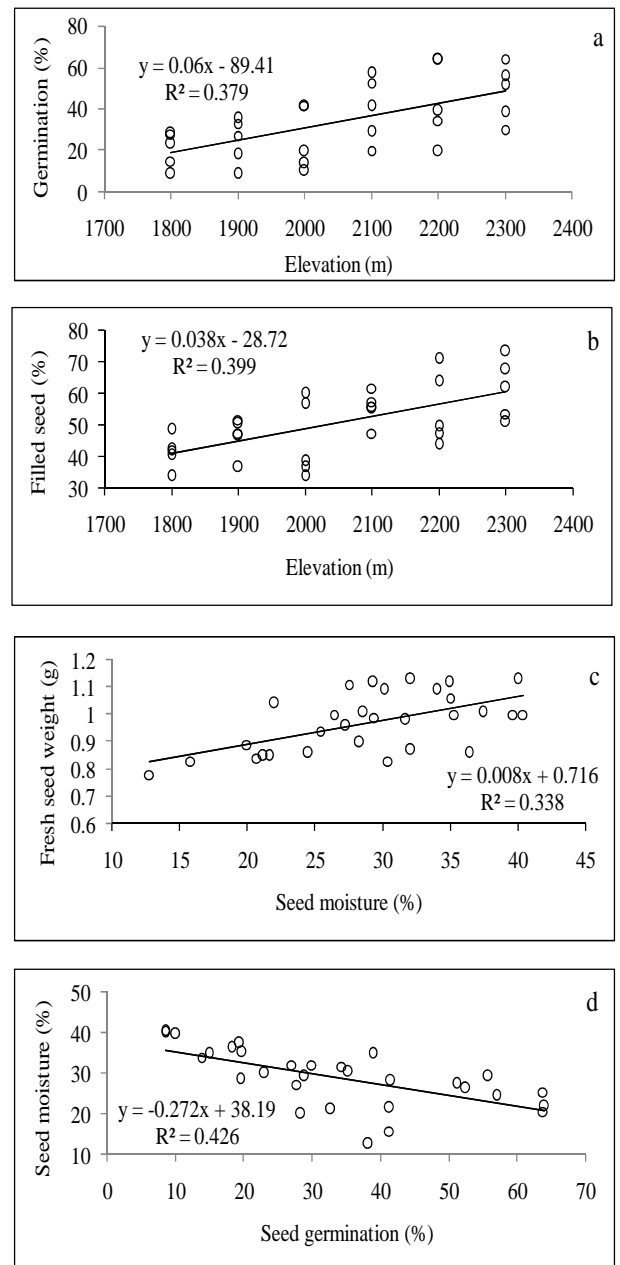
Seeds of 1<sup>st</sup> years were larger in size, heavier in weight, low in moisture percentage with higher seed germination percentage (Fig. 2 a-d). ANOVA indicated that seed weight ( $f=609.7$ , 1df), seed moisture ( $f=676.3$ , 1df), seed germination ( $f=272.0$ , 1df) were highly significant between the years except seed size ( $f=0.08$ , 1df). Paired t- test between the years indicated that fresh seed weight ( $t_{0.01}=4.6$ , 4df), seed moisture percentage ( $t_{0.01}=5.7$ , 4df) and seed germination percentage ( $t_{0.01}=3.8$ , 4df) varied between the years. Seeds in the year 1<sup>st</sup> above 2000 m elevation mature one week early compared to below 2000 m, while no such variation was observed in year 2<sup>nd</sup>.

ANOVA indicated that the seed size ( $f=2.58$ , 4 df), fresh seed weight ( $f=203.7$ , 4df), seed moisture percentage ( $f=1310.0$ , 4df), filled seed percentage ( $f=64.0$ , 4df) and seed germination percentage ( $f=287.5$ , 4df) significantly varied among the collection dates. Seed fresh weight and moisture decreased significantly whereas germination, filled seed percentage increased significantly as the collection progress. Seed weight

was 1.05 at the beginning to 0.84 g/100 seeds at the end. Seed moisture decreased from 37.4 at the beginning to 18.7 % at the end. Seed germination increased from 16.9 to 41.0 %, filled seed from 44.3 to 54.6 % at the end of collection across the elevation. Seed moisture at maturity ranged between  $22.3 \pm 2.0$  and  $30.4 \pm 0.8$  % among the elevations. Fourth collection indicated the peak germination (48.2 %) at moisture 26.3% and may be the appropriate time for seed harvesting. LSD between the collection dates indicated that seeds size varied between 16 Oct and 7 Nov; and between 7 Nov and 15 Nov. Seeds of 7 Nov, and 15 Nov had significantly lower in weight compared to first three collections. Seed moisture varied from one collection date to another. Similarly, seed germination also varied from one collection to another except between 23 Oct and 30 Oct.



**Figure 1** Variation in different seeds parameters in different collection dates. Collection date 16O denotes October 16; 23O, October 23; 30O, October 30; 07N, November 07 and 15N, Nov 15. Dark shaded bars represents year 1<sup>st</sup> and light shaded bars year 2<sup>nd</sup>. Error bars indicate  $\pm 1$  se.



**Figure 3** Relationship between (a) Elevation and germination percentage (b) elevation and filled seed percentage (c) seed fresh weight and seed moist (d) seed moisture and germination percentage of *C. viminea*.

## DISCUSSION

Today, the whole world is concerned about to preserve the ecological balance and heritage of earth in changing climatic conditions. Under such situations tests of seed maturity indices in relation to climatic variables may be used to predict the adaptiveness of a species, especially the under canopy. Considerable variations in *C. viminea* were found in seeds traits and germination behavior between the years, among the elevations and collection dates. Seed fresh weight, germination percentage and filled seed production increased with increase in elevation while seed moisture percentage declined. Increase in germination percentage and rate with increasing elevation may be due to higher gene flow above 2000 m elevations. The species is distributed between 1800 and 2400 m elevation in the

Himalayas and elevations 2100 and 2200 m are the central zone of its distribution from where the species migrated. Below and above from these elevations are the peripheral zones and the species migrated from there. Studies indicated that peripheral populations may be more susceptible to the stress imposed by climate change due to their already compromised fitness and reduce genetic variation, especially if competition with other species limits their ability to migrate (Aitken *et al*, 2008). However, a warming climate would be expected to cause relatively less stress to peripheral populations that inhabit the high-latitude or high-elevation leading edge of a range, because gene flow from central populations would introduce alleles pre-adapted to a warmer climate (Davis and Shaw, 2001). The decline in temperature from 1.5 to 4.7 °C in different months and annual rainfall from 59.8 to 151.2 mm differentiate the climate of 1800 m and 2300 m elevation. Elevation showed a significant positive relation with germination (Fig. 3a) and filled seed production (fig. 3b). Bhatt and Ram (2005) concluded that greater germination at high elevational seeds may be attributed to seed weight and sound seed percentage. In both the year seed moisture decreased and germination increased in with decrease in elevation. Earlier decrease in temperature together with frost and dew may be responsible for earlier drying of seeds at higher elevations.

All the seed traits, except seed size, studied were varied significantly between the years. This yearly variation in seed traits can influence the regeneration. Seeds of year 1<sup>st</sup> were greater in fresh weight, germination, moisture content and filled seed production. This may be due to variation in temperature and rainfall that change the flowering phenology of the species. Increase in temperature (0.67°C January) responsible for one week earlier leafing and flowering earlier in 2<sup>nd</sup> year which may adversely affected pollination success and could be one of the reason for low filled seed percentage consequently the germination. Production of seeds with different viability is one of the most important survival strategies for species growing under unpredictable environmental conditions (Gutterman, 1994; Kigel, 1995). Seeds attained their full size and remains on the trees till seed fall regardless of filled or empty. Shibata *et al*, (1998) reported that seed of four co-occurring *Carpinus* species was attained their full size regardless of successful fertilize in Japan.

Seeds in 1<sup>st</sup> years of 2000 m and above mature (maximum germination) one week early compared to 2000 m and below, whereas no such variation was found in 2<sup>nd</sup> years. Climatic variations may be responsible for different degree of physiological stages of seeds at different elevations. Decline in 0.96 °C temperature below 2000 m could be responsible for simultaneous maturation of seeds in year 2<sup>nd</sup>. Bracts changed their color from green to yellowish brown and seed coat from green to grayish brown as the collection progressed. These morphological features can be used as a reliable ocular indicator of seed maturity. Hori and Tsuge (1993) found that photosynthetic rate of the bract of *C. laxiflora* decrease after August to mature the seeds. Dirr (1990) and Hartmann *et al* (1990) reported that the best seed coat colour for collection of European Hornbeam (*C. betulus*) seed is green (when the wings are turning yellow and are still soft and pliable). Many researchers has also developed relationships between colour

changes and maturity, because of its more practical utility than moisture, germination etc. which requires time and laboratory facilities.

Seed attained full size prior to maturity as no clear trends was observed among the collection dates. This indicates seed size has no bearing in the maturation. Seeds lose their fresh weight as the collection progressed. Fresh weight showed positive relationships with seed moisture (Fig. 3c) and seed moisture negative with seed germination (Fig. 3d). This is consistent with maturation drying and may be attributed to rapid water loss, physiological and chemical changes in the seeds, so that they mature and developed viability for longer period to maintain their populations. Best seed germination was found between 22 and 30% seed moisture at the studied elevations. At peak germination ranged between 39.3± 3.5 and 70.0± 2.0 % and filled seed between 40.5± 3.0 and 73.5± 4.1 % across the elevations. Seed moisture showed negative relation with germination. Such relations were also reported for other deciduous species *Populus ciliata* (Pandit *et al*, 2002), *Pyracantha crenulata* (Shah *et al*, 2006) and *Prunus ceresoides* (Tewari *et al*, 2011).

From the study it was found that Himalayan Hornbeam differs from its counterpart of Europe and America in growth and germination behavior. Its germination was 48 % which is higher compared to 30 % *C. betulus* and 2 % *C. coroliniana* (Rudolf and Phipps, 1974), though, the sound seed percentage was similar (ca 60%) in these species. Low germination percentage in *C. betulus* and *C. coroliniana* is attributed to seed dormancy. ISTA (1993) recommended one month moist incubation at 20°C followed by chilling (3-5°C) treatment for four months for better germination of *Carpinus* species. Pipinis *et al*, (2012) reported that 70% germination was observed in warm and cold stratification and gibberellic acid treatments in *C. betulus* and *C. orientalis* seeds. Seed dormancy may be related to the growing environmental conditions and genetic makeup of the species. *C. betulus* and *C. coroliniana* are growing relatively cold climate compared to the distribution range of *C. viminea* in the study area. Therefore, Himalayan species produced less dormant seeds. The Himalayan species was taller (18.9 m) in height compared to *C. betulus* (12.2 m) and *C. coroliniana* (6.9 m). Contrasting growth and seed germination behavior of *C. viminea* from its allied counter part of Europe and America supports the view of Zobel and Singh (1997) that forest in the Himalaya are different from temperate forests of the world.

## CONCLUSION

The study showed that seed maturation period and germination percentage varied significantly between the years. Seed maturity period lies in between 1<sup>st</sup> and 2<sup>nd</sup> week of November and seed germination depends on sound seed production. The elevations 2100 to 2300 m are the potential sites for seed collection. Rise in temperature in the month of January induces early leafing and flowering and high rainfall at the time of pollination adversely affects its pollination success, and could be one of the reasons for low sound seed percentage between years. Such erratic climatic patterns likely to be more pronounced in coming future due to climate change therefore,



the existence of the species below 2000 m in jeopardy. However, more data are required to make a sound decision.

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