

Seed germination behaviour of *Quercus leucotrichophora* (Banj oak) in Western Himalaya

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ABSTRACT

Quercus leucotrichophora A.Camus (Banj oak) is one of the keystone species in the mid-elevation forests across western and central Himalaya. Its regeneration in many parts is reported to be poor due to low germination and seedling emergence. Present study aims to investigate the germination behaviour of Quercus leucotrichophora. Seeds were collected from 5 different sites and sown in polybags under open, poly-house and shade-net-house conditions. Seed germination started in 20 days and was completed in 92 days. Higher seed germination was observed in poly-house conditions (76.66%-82.66%) followed by shade-net-house conditions (68.23-76.66%), while, minimum germination was observed in seeds placed under open conditions (61.66%-74.33%). Mean Germination Time was found rapid in poly-house conditions (8.4-10.13) followed by shade-net house conditions (8.79-12.71) and open conditions (20.39-24.66). Among all the sites, higher germination was recorded for the seeds collected from the mid altitude regions (1300-1400 m asl). A significant positive correlation (P<0.05) between seed size class with cumulative germination percentage was also noticed. The findings of the present study indicated that, site of seed collection can play a crucial role in seed germination. Thus, for raising quality planting material of Q. leucotrichophora, seed collection should be done from specific habitats that ultimately help in restoring the declining population.

INTRODUCTION

Himalayan oaks are generally regarded as late-successional trees (Singh & Singh, 1987; Fartyal et al., 2022). In the Himalaya, *Q. leucotrichophora* A.Camus ex Bahadur (Banj oak) is dominant evergreen forest species with massive root system, (Bargali et al., 2014, 2015) and is among the five evergreen oak species of the Central Himalayan forests (Bargali & Singh, 1996). *Quercus leucotrichophora* is a medium sized commonest oak of the western Himalaya and plays a pivotal role in ecosystem services by providing green Leaf-fodder and quality fuel wood and ecological services by conserving soil and water (Bisht et al., 2013). Forests dominated by the *Quercus* (Oak; family Fagaceae) are considered established and climax communities (Singh & Singh, 1986), however, are very prone to climate change (Bisht & Kuniyal, 2013). Banj oak is distributed between 800-2300 m elevations and serves

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as a 'keystone' species in the region (Zobel, Ram & Bargali, 1995; Bisht et al., 2021). Simultaneously occurring leaf fall and emergence of new leaves, overall growth and development, regeneration and distribution of oaks in their specialized habitats are governed by the ambient conditions (Bisht & Kuniyal, 2013). Spatial distribution and oligarchic nature of the *Quercus* is a peculiar and adaptive phenomenon (Bisht & Kuniyal, 2013). The wood is used as fuel and for making agricultural tools, leaves as green fodder particularly during lean periods and leaf litter as cattle bedding. Acorns (seeds) make wildlife food for animals like Monkeys (*Macaca mulatta*), Asiatic black bear (*Selenarctos thibetanus*), Giant flying squirrels (*Petaurista petaurista*), etc. and many species of birds (Bargali et al., 2015).

Seed is the natural resource by which species propagated and flourished, however, natural regeneration of a species depends upon the production and germination capacity of the seeds and the successful establishment of seedlings (Rao, Ralhan & Singh, 1986). Emergence and establishment of seedlings in the oaks under their habitat conditions are reported unsatisfactory (Bisht & Kuniyal, 2013). Viviparous nature, susceptibility of seeds to frugivory, inadequate and interrupted regeneration, low seedling establishment and trampling driven damages to seedlings by browsing animals are attributed as key reasons for degradation of Oak forests (Bisht et al., 2013). If the mast fruiting year is not accompanied by satisfactory precipitation, the viviparous species may lose the opportunity for regeneration due to desiccation or water stress. Massive lopping for fodder, habitat degradation are other considerable factors for poor seed production and regeneration of oaks in the mountain regions (Pandey, Bargali & Bargali, 2017). In future, due to meagre regeneration and climate change, it will be merely possible to find the identical oak forest replaced by its next generation (Bisht & Kuniyal, 2013). Despite its great importance, the efforts for its regeneration, seed germination and seedling establishment are meagre (Bargali & Bargali, 2016). It is well known that population of different geographical locations showed area-specific responses in their phenology. These forests are important for inflow and spring recharge of water for drinking and domestic uses of local residents (Manral et al., 2020, 2022; Bisht et al., 2022) In order to meet the needs, the species is being harvested at an alarming rate from the natural populations. The future of these forests depends upon the regeneration and successful establishment of seedlings.

Therefore, present study was carried out to see the germination behavior of *Quercus leucotrichophora* populations collected from different locations in western Himalaya.

MATERIALS AND METHODS

Seed Collection: Mature and seemingly healthy seeds were collected from five different locations, i.e., Agrora, Kandoliya and Khirsu from Pauri district, Langasu from Chamoli, and Mayali from Rudraprayag district of Garhwal Himalaya during the winter season of 2020-2021, i.e., during the time of peak seed fall (Table 1, Figure 1). Collected seeds were carried to the laboratory in polythene bags for measurement of seed length, diameter, weight, moisture etc. Seed length and width of seeds with acorn and without acorn were measured using Vernier Calliper, seed weight was measured using analytical balance (error: 0.002 mg); and seed moisture was determined by drying the seeds at $105\pm2^{\circ}$ C in a pre-set incubator until the constant weight. Each replicate consisted of 20 seeds with four replications.

The experiment was conducted at Department of Natural Resources and Forestry, H.N.B. Garhwal University, Srinagar (Garhwal), Uttarakhand. Geographically, the experimental site is situated in Alaknanda valley which lies between 78°47'30" E longitude and 30°13'0" N latitude, at an elevation 540 m above msl., in the lesser Himalayan region. Study area has a sub-tropical climate, with extremes in the temperature, i.e., fairly cold winter and very hot summer. The temperature sometimes goes as low as 5 to 9°C in the month of December to January and up to 35-40°C in the month of May to June. During winter, frost and during summer, hot scorching winds are common. Nearly 80% of the total rainfall is received during the monsoon with few showers in the winter. During the experimentation, the mean

monthly temperature ranges from 20.8°C to 35.92°C in the experiment site. The rainfall is around 1437 mm. The meteorological data of the study area is presented in table 2.

Seed Viability Assessment: To ensure the seeds used for the experiment were viable, the sample lots were subjected to viability test using 0.5% (w/v) tetrazolium salt (TTC) (2, 3, 5-triphenyl tetrazolium chloride) solution. Four replicates of freshly harvested 30 seeds each were subjected to TTC test under controlled dark conditions for 24 h at 25°C. Subsequently, each seed was dissected and a completely red stained embryo was considered a viable seed (Bisht et al., 2017). The seeds were stored at 25°C at room temperature for 2 months to check the viability up to 2 months.

Seed Imbibition Assessment: To verify if the species have water impermeable seed coat, four replicates of 20 seeds each for each location were weighed and allowed to imbibe water in a beaker (200 ml) at room temperature (25°C) under dark condition. At regular intervals (2 h) seeds were removed, blotted dry, weighted and were put back to beaker containing water. The process was repeated till the seed weight remained constant (Baskin & Baskin, 1998). The amount of water imbibed by the seeds is presented as the percent increase over the initial weight and was calculated as:

Imbibition =
$$\frac{Fresh weight - wetweight}{Fresh weight} x 100$$

Pre-sowing seed treatment: Freshly collected seeds were surface sterilized with 0.2% mercuric chloride $(HgCl_2)$ for 2 min to remove any fungal infection, followed by rinsing with distilled water. Four replications, each of 20 seeds were prepared for seed germination study. The seeds were sown in polybags (22 cm x 9 cm) having soil, farmyard manure and sand in the ration of 1:1:1. Germination was monitored every day for 60 d. Seeds were considered germinated when visible protrusions of plumule were observed. The final germination was considered after recording constant readings for 15 days. The data were presented as percentage of the data accumulated.

Data Analysis: Germinability (G) or percent of germinating seeds; time to first observed germinant (T) in days (d) and time to maximum germination (T_{100}) in days were calculated. Seed germination was calculated as:

Germination % =
$$\frac{Total \ number \ of \ germinate \ seeds}{Total \ number \ of \ seed \ sown} x \ 100$$

Mean germination time (MGT) was calculated following Kochankov et al. (1998) as:

$$MGT = \frac{\Sigma(fx)}{\Sigma x}$$

where x is the number of newly germinated seeds on each day and f is the number of days after seeds were set to germinate. Germination rate (GR) was calculated following Rossello and Mayol (2002) as $M_a/(M_g - I_g)$, where, M_a is maximum number of germinant, M_g is days to maximum germination and I_g is days to initial germination.

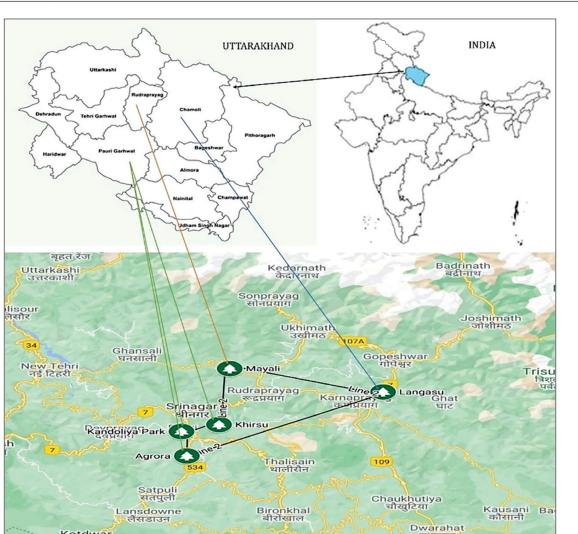


Figure 1. Study area

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S.No.	Name of the site	Altitude (m asl)	Longitude (N)	Latitude (E)
1	Agrora	1345	30°05'69"	78°78'59"
2	Kandoliya	1698	30°14'71"	78°77'17"
3	Khirsu	1409	30°17'22"	78°86'80"
4	Mayali	1339	30°37'89"	78°89'56"
5	Langasu	900	30°29'30"	79°28'88"
	(

RESULTS AND DISCUSSION

Mean highest seed length (mm) was observed for the seeds collected from Khirsu with average seed length of 25.88 ± 0.59 followed by Mayali (24.88 ± 0.45) while was minimum for the seeds collected from Agrora (22.77 ± 0.77). Mean highest seed width (mm) was recorded for the seeds collected from Khirsu (15.84 ± 0.23) followed by Mayali (15.66 ± 0.78) and was observed minimum for Agrora (12.67 ± 0.32). Average highest seed weight was recorded for the seeds collected from Khirsu (20.02 ± 0.90), followed by Mayali (19.26 ± 0.63). However, the lowest seed weight was observed for seeds collected from Agrora (18.22 ± 0.90) (Figure 2). Higher mean acorn size in seeds collected from Khirsu and Mayali may be attributed to

their favourable climatic conditions in these altitudinal range (Figure 2). It is reported that, mean acorn size was larger at the lower elevation site than higher elevation site across the collection dates (Tewari, Mittal & Singh, 2017). However, in present study, we observe the long acorn size at middle attitude (Khirsu; 1409 m asl) followed by Langasu (900 m asl) and going down the altitude or up the altitude has negative impact on the

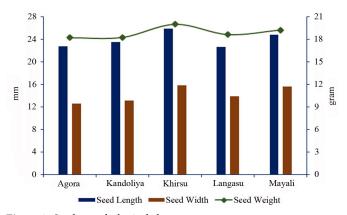


Figure 2. Seed morphological characters

 Table 2. Monthly meteorological data during the period of experiment

 2020-2021

Month	Temperature (°C)		Relative humidity(%)		Rainfall (mm)	
	Min.	Max.	Min.	Max.		
December, 2020	11.51	12.91	66.15	72.13	1.8	
January, 2021	11.68	13.07	68.85	72.69	15.8	
February, 2021	16.08	17.59	57.34	63.84	14	
March, 2021	21.39	23.32	40.86	47.02	37.4	
April, 2021	24.04	25.68	34.95	41.29	102	

Source: Department of Rural Technology, HNBGU

seed size. This may be attributed to environmental and maternal condition operating at this altitudinal range, which may favour the optimum seed development, efficiency of new seedling establishment and survivorship (Murali, 1997). However, resource availability may also play a crucial role in seed morphological characters and therefore, may influence the seed size (Murali & Sukumar, 1994). The bioclimatic circumstances of Himalayan landscapes alter very rapidly within a very short distances which articulate heterogeneity of soil types as well as their properties (Bargali et al., 2018; Padalia et al., 2022) which may affect the plant characteristics (Bargali, Padalia & Bargali, 2019).

Seed viability: 0.5% (w/v) tetrazolium salt (TTC) test showed that more than 80% viability of seeds at the time of seed collection for different sites having highest viability in seeds collected from Langasu (96.56±5.09), followed by Mayali (89.65± 5.07), Kandoliya (88.92±6.9). However, lowest viability was recorded from Khirsu (86.68±1.8). After one month, the

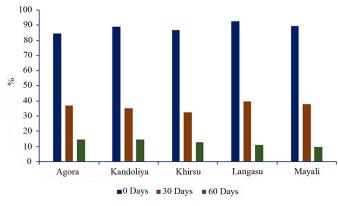


Figure 3. Decrease in seed viability of *Q. leucotrichophora* during storage at room temperature

seed viability was found highest for Langasu (39.45 ± 1.6) followed Mayali (37.82 ± 1.2), Agrora (36.98 ± 2.1), while was found lowest for Khirsu (36.65 ± 1.8). Likewise, after 02 months, the seed viability was found highest for Agrora (18.52 ± 3.2) followed Kandoliya (14.65 ± 2.6) while was lowest for Mayali (9.56 ± 2.8) (Figure 3). In present study, it was found that the seeds lose its

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viability by more than 50% within one month which was reduced to less than 15% in 02 months of storage at room temperature. It is reported that seed viability reduces fast when seeds were stored at room temperature which is also found comparable with the findings of the present study. Troup (1921) reported that the viability of the seeds of *Q. leucotrichophora* can retain up to 12 months with a fertility of 60 percent if carefully stored in cool dry place and protected from insect attack. The results obtained in this study have indicated that seed storage even for a period of 60 days under ambient conditions losses its viability, thus, has negative impact on standard seed germination. Present study found that seed moisture content and seed viability decreased with storage time.

The moisture content of oak acorns has been proposed as an indicator of viability and hence germination (Bhatt & Ram, 2015). Edwards (1980) reported that fleshy fruits gain moisture with ripeness, while dry fruits tend to lose moisture with maturity. Similar results were obtained in this study, with

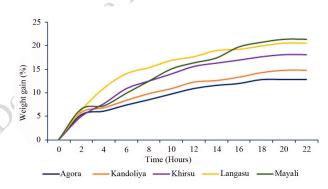


Figure 4. Seed imbibition of Q. leucotrichophora

maturity there is a decrease in moisture content of acorn. The ideal seed moisture content should be in the range of 38 to 45%. Joshi, Tewari & Ram (2022) also found highest germination in seeds having moisture content between 35 to 41.7%. Seed loses their weight as the collection progressed due to loss in moisture content (Bhatt & Ram, 2015). The seeds of the Oak died when their moisture content drops below 30% to 35% (Connor, 2004), thus, confirming the recalcitrant nature of *Q. leucotrichophora* seeds. The correlation coefficient between the seed size and the moisture content was found negatively correlated (P < 0.05).

Seed Imbibition: Imbibition of water by the seeds was very fast in the first 2h and then it increased gradually. The average increased weight (%) by imbibition was found highest in seeds collected from Mayali followed by Langasu, Khirsu, Kandoliya while it was found lowest in Agrora. The average increase in weight (%) ranged between 4.96 to 6.51 in the first 02 h of imbibition, while after 22 hours of imbibition the increase in seed weigh was recorded to be 12.88 to 21.30. Furthermore, no considerable increase in weight was observed (Figure 4).

Seed Germination (%): The seed germination started from 20 days and almost completed by 92 days. Highest seed germination in all the population were observed in poly-house conditions followed by shade-net house conditions while,

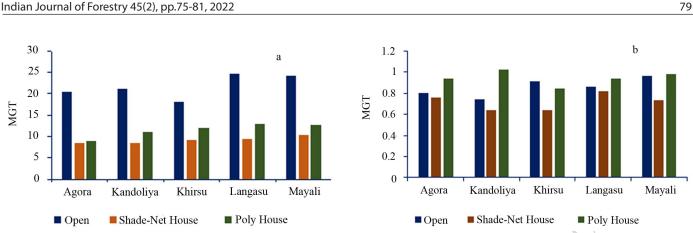


Figure 5. a - Mean Germination Time (MGT); b – Germination Rate (GR) in Q. leucotrichophora

Table 3 Germination behaviour of seeds of (. leucotrichophora collected from different location in different seed sowing	treatments
Table 5. Germination benaviour of seeus of Q	<i>ieucon unopilora</i> conected nom amerent location in amerent seed sowing	2 treatments

Seed sowing parameters			Germination %		
	Agrora	Kandoliya	Khirsu	Langasu	Mayali
Open	68.33±2.25	61.66±0.46	74.33±1.44	71.66±3.02	69.66±1.12
Poly House	76.66±1.66	71.66±1.62	82.66±1.63	79.33±2.14	76.66±1.22
Shade Net House	68.00±0.36	65.23±1.22	76.66±0.92	75.33±0.69	73.33±2.32

minimum germination was observed in seeds placed under open conditions. In poly-house conditions, the seed germination was ranged between 76.66% (Agrora and Mayali) to 82.66% (Khirsu). Likewise, in shade net house condition, the germination was found highest for Khirsu (76.66%) while it was lowest for Kandoliya (65.23%). For open conditions, the germination was found between 61.66% (Kandoliya) to Khirsu (74.33%) (Table 3). A significant variation was observed in seed germination behaviour along with different seed germination treatment (p = 0.05). Highest germination in all three treatments (open, poly house and shade net house conditions) was observed for the seeds collected from the Khirsu region followed by seeds collected from Mayali regions. These two regions fall in mid altitude range (1300 to 1400 m asl) and Q. leucotrichophora is well acclimatized to this altitude. Thus, higher germination in seeds collected from these two locations may be an adaptive feature of the species. We also observed significant positive correlation between seed size class with cumulative germination percentage (P<0.05). Earlier reports also indicated a significant positive correlation between seed size with germination (Karki, Bargali & Bargali, 2018). The findings of the present study thus indicated that, seed collection from different sites can play a crucial role in seed germination and seedling establishment. Thus, for raising quality of planting material of Q. leucotrichophora, seed collection from specific habitats can be promoted, this will also enhance the germination procedure and thereby speed up plantation programs (Das, 2014; Nautiyal, Rawat & Prasad, 2000).

Mean Germination Time (MGT) was found fast in poly house conditions followed by shade net house conditions and was slowest in open conditions. In open conditions, MGT was ranged between 20.39 (Agrora) to 24.66 (Langasu), while for shade net house condition, it was ranged between 8.79 (Agrora) to 12.71 (Langasu). Likewise, for poly house conditions, it was between 8.4 (Agrora) to 10.13 (Mayali) (Figure 5a). Germination of the seeds of Q. leucotrichophora is hypogeal with the nut splitting towards the apex and the radical emerging and growing downward to form a young taproot. Similar finding was recorded in C. australis (Singh, Bhatt & Prasad, 2004). Germination Rate (GR) was found fast in poly house conditions followed by shade net house conditions and was slowest in open conditions. In open conditions, GR ranged between 1.08 (Mayali) to 1.23 (Kandoliya), while for shade net house condition, it was ranged between 0.92 (Langasu) to 10.6 (Kandoliya). Likewise, for poly-house condition, it was between 0.84 (Khirsu) to 1.02 (Kandoliya) (Figure 5b). The delay in germination was because of hard seed coat. Generally, germination in Quercus spp. was reported to decreases with increasing elevation. The sharp decline in temperature in November and onwards may affect the proper development of seeds at higher elevation which may be responsible for low germination; mild temperature at mid and low elevation favours the seed development, as a consequence of this, seeds attain their large size and weight in these elevations. Reproductive phenology such as seed formation, seed dehiscence, seed germination and seedling establishment in Quercus are triggered with the commencement of favourable growth season and a small shift in these conditions in a unit area and at a specific time may be indeterminate variable for driving phenology, growth and reproduction of Quercus (Bisht & Kuniyal, 2013; Bisht et al., 2013; Karki, Bargali & Bargali, 2018).

The seed morphology in *Q. leucotrichophora* make it incompatible with upward or wind-aided movement (Bisht & Kuniyal, 2013) infect are design to dwelling-down due to round or conical acorn. Despite that, *Q. leucotrichophora* is rarely observed to flourish at lower altitudes. Therefore, the acclimatization of *Q. leucotrichophora* in their specialized

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habitats may rarely follow the variation in ambient conditions. Because the oaks are well adapted to their peculiar growth habitats, seed production strategy and germination behavior is synchronized with this specialized habitat. Destruction of this specialized habitat, degradation of this ecologically important species and replacement colonization of uncertain and unknown species will certainly alter future ecological functioning of the present oak forest and thus also impact the ecosystem services provided by the Oaks. Protection and maintenance of oak forests, planting of seedlings in their specialized habitats, and preventing movement of grazing animals in these specialized habitats during seedling establishment may be useful in conserving these specialized habitats.

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