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CONSERVATION STRATEGIES FOR THE THREATENED *QUERCUS SEMECARPIFOLIA SM.* A HIMALAYAN TIMBERLINE OAK

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Abstract

Quercus semecarpifolia Sm. is a temperate, timberline, multipurpose oak species. It plays an important role in the Himalayan ecosystem. Human pressure, predation, short-lived acorns, failure of good seed crop every year, poor regeneration, desiccation, frost sensitivity and climate change may lead to complete extinction of the species in a near future. Immediate attention to conservation and restoration measures of the Himalayan ecosystem, in which this species grows, is required. Thus, an experiment was designed to analyze the role of planting stock and micro-sites for the regeneration of Q. semecarpifolia in-situ. For that purpose, 16 microsites with presence/absence of litter, canopy and sunlight were identified, where seeds/propagules were planted. The presence of litter, canopy and sunlight influenced the seedling production, mainly impairing the processes, leading to the expansion in diameter of the radicle, causing shoot/root emergence. Radicle emergence took place in all the seeds immediately after shedding but only 20% of seedlings recovered; however, when the stored seeds were sown as soon as the emerging radicle attained 10-15cm in length and 2-3cm in diameter, 80% of seedlings were recovered. Surprisingly, after being detached from the seed the swollen radicle acted as a propagule and produced 80% seedlings irrespective of the microsites; however, radicles detached prior to diameter expansion failed to produce seedlings which is a crucial insight for promoting seedling production.

Keywords: *Q. semecarpifolia; in-situ* regeneration; microclimate; conservation; ecosystem restoration; radicle autonomy.

Introduction

Q. semecarpifolia Sm. (brown oak, kharsu) is a timberline, dominant oak species of the Western Himalayan region, found in moist areas in the temperate and sub-alpine areas. *Q. semecarpifolia* forests are not only important from the ecofloristic point of view: they are also part of the lives of people living in the area. Indeed, it is a multipurpose tree used for fodder, fuel-wood [1], wood for agricultural implements and tannin [2, 3]. Because of high human pressure [2, 3, 4, 5] and predation [3, 5, 6], forests degenerated in areas where the species was abundant, which may lead to a dramatic expansion of xerophytic conditions and possibly to the complete extinction of the species [7, 8]. If such a trend continues the population may disappear

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from the Himalayas in the near future [9], threatening the Himalayan ecosystem [3, 8]. The Convention on Biological Diversity founded in 1992 identified biological invasion, habitat destruction and fragmentation, as the worst threats to biodiversity [10]. It was also predicted that climate change will remain one of the major drivers of biodiversity patterns in the future [11, 12] and our study area is not untouched by those factors, thus immediate attention is required to find efficient and low-cost means to conserve this threatened timberline ecosystem.

Conservation of the species depends mainly on the sustainable utilization as well as on regeneration potential in situ [13]. Studies on the natural regeneration dynamics of *Q. semecarpifolia* have not been done so far in the projected area, but some other studies also highlighted the constraints of the regeneration in situ [8, 9, 14]. Successful *in-situ* regeneration requires appropriate natural conditions, the establishment of vigorous seedlings and their subsequent survival. Successful seedling establishment mainly depends on proper planting stock and on the environmental conditions of the planting site [5]. The role of proper planting propagule should not be ignored. They require an understanding of seed characteristics and germination behavior.

Previous studies on seeds of *Q. semecarpifolia* indicated that very high moisture content and the short viability of the seeds, along with failure of a good seed crop every year, desiccation and the frost sensitivity of seeds, their low regeneration potential in natural conditions, edibility of seeds by wild animals, heavy human interaction and climate change pose a serious problem for the regeneration of its genetic resources and endanger diversity [2, 5, 15].

Seed germination and seedlings establishment in *Q. semecarpifolia* differs from associated species and other oaks [5, 14]. Radicle emergence in this species takes place immediately after seed shedding, but shoot emergence and seedling establishment takes more time [14]. The radicle attains maximum length (up to 10-15cm) within one month, followed by a 2-3cm thickening of radicle diameter (swollen radicle) within one month. Most of the seeds remain in that condition until the next growing season and in the subsequent year the shoot emergence from this swollen portion takes place, after a longitudinal split and the rest of the radicle acts as a root when put into the soil and then secondary roots emerge. Later on, the remaining portion of the seed gradually degenerates. It can be presumed that this swollen radicle is a part of an adaptive strategy to frost tolerate and desiccation, in contrast to the whole seed [5]. Based on that assumption, regeneration studies were carried out using seeds with swollen radicles and useful results were obtained.

According to our understanding of seed characteristics, germination and seedling establishment, forest status, local dependency, the ecological and economic value of the tree, we conducted the study with an aim to determine the causes for the reduction in regeneration potential and population size of *Q. semecarpifolia* in the area. We aimed to help maintain the diversity, ecological balance, livelihood and economy of the area for a sustainable utilization through restoration of this oak species in its natural habitat, so as to preserve the fragile Himalayan ecosystem.

Materials and methods

We conducted studies with the seeds (acorn *sensu stricto*) of *Q. semecarpifolia* (brown oak, kharsu) collected from the forest floor under the canopy of healthy trees, growing in their natural stand, near Duggalbitta (2590 m). It is located between 77^0 33' 5" 80^0 6' Eastern longitude and from 29^0 31' 9" to 31^0 26' 5" Northern latitude, in the Rudraprayag district, Uttarakhand, India. To maintain local genetic integrity, the seeds were collected from the same forest where the regeneration studies were conducted, for the restoration of the species. For this purpose, the forest floor was first swept, to remove earlier fallen seeds and after 24 hours we collected the seeds abscised during this period. The acorns were then immersed in water to remove leaves, cups, other debris and insect damaged acorns that float. Only healthy and mature acorns were used for We considered that mature seeds were those easily sloughing out of their outer caps, called cups or cupule.

High predation rate and anthropogenic interaction inhibit many acorns from producing a viable seedling, so to protect the acorns; the experiment area was well fenced. The microsites where the seeds were placed were covered with iron angles surrounded by an iron mesh which also protected acorns from being displaced during heavy rainfalls.

The natural habitat is comprised of different microsites conditions. The understanding of the role of microsites in seedling recruitment plays a key role in the future development of the forest and in its composition [16]. Thus, different microsites were identified in the natural habitat of *Q. semecarpifolia* and observations were made for a regeneration pattern**.** To test the regeneration potential of *Q. semecarpifolia*, mature seeds/planting propagule were placed in square intervals of 5X5 m *in situ,* in different microsites representing a variety of habitat conditions. The seeds/propagules were placed to regenerate over the forest floor, similar to natural seeding conditions, so as to assess their natural regeneration potential.

Immediately fallen seeds, as well as seeds which had been stored for one or two months, were used as the planting stock for the *in situ* regeneration. As with many oaks, radicle sprouting cannot be restricted during storage [17] and within one month of storage some seeds develop a radicle with a maximum length of 10-15 cm and a thickness of approximately 0.5 cm. During two months storage that full length radicle expands in diameter (approx. 2cm) almost at the middle of the radicle. The length of this swollen portion is around 4-5 cm.

Freshly fallen mature seeds (FFMS in A1 to A4 microsite conditions), seeds stored for one month, with a full length radicle (SFLR in B1 to B4 microsite conditions), and two months stored seeds, with a swollen radicle (SSR in C1 to C4 microsite conditions), detached swollen radicles their expansion in diameter (SRD in D1 to D4 microsite conditions), were placed in different microsite conditions *in-situ,* in the month of July, August and September, respectively. A total of 16 microsite conditions were identified, as presented in Table 1, below.

Those microsites consisted of:

- (A1) freshly fallen mature seeds (FFMS) sown under the canopy with litter;
- (A2) FFMS seeds sown under the canopy, without litter;
- (A3) FFMS seeds sown outside the canopy, with litter;
- (A4) seeds sown outside the canopy without litter;
- (B1) seeds stored for one month, with a full length radicle, before swelling (SFLR) were sown under the canopy with litter;
- (B2) under the canopy without litter;
- (B3) outside the canopy with litter;
- (B4) outside the canopy without litter;
- (C1) **s**eeds stored for two months, with a swollen radicle (SSR) were sown under the canopy with litter
- (C2) under the canopy without litter;
- (C4) outside the canopy with litter;
- (C3), outside the canopy without litter
- (D1) swollen radicles detached from seeds (SRD) were sown under the canopy with litter
- (D2) under the canopy without litter;
- (D3) SRD were sown outside the canopy with litter;
- (D4) SRD were sown outside the canopy without litter.

Full length radicles detached from the seeds prior to swelling, were also placed under different microclimatic conditions, as mentioned above, but in all the conditions, all the radicles deteriorated/dried in 3-4 days (not detailed here). Partial light was available on the ground, under the canopy of *Q. semecarpifolia,* but outside the canopy there was overhead light**.**

Previous studies indicated that the detached swollen radicles can act as a propagule for the regeneration of *Q. semecarpifolia* [5]. Thus in the conditions D1 to D4, detached swollen radicles were sown.

Table 1. Different microsite conditions and the planting stock used to assess the regeneration potential of *Quercus semecarpifolia* in its natural habitat

S.N.	Planting Stock	Abbreviations	Litter and canopy conditions under different microsites *in situ*				Month of Sowing
			With Litter	Without Litter	Under the Canopy	Outside the Canopy	
1.	Freshly fallen mature seeds (FFMS)	FFMS (A1)	√		√		July
2.	-----do-----	FFMS (A2)		√	√		July
3.	-----do-----	FFMS (A3)	√			√	July
4.	-----do-----	FFMS (A4)		√		√	July
5.	One month stored seeds having full length radicle before swelling (SFLR)	SFLR (B1)	√		√		August
6.	-----do-----	SFLR (B2)		√	√		August
7.	-----do-----	SFLR (B3)	√			√	August
8.	-----do-----	SFLR (B4)		√		√	August
9.	Two months stored seeds, with a swollen radicle (SSR)	SSR (C1)	√		√		September
10.	-----do-----	SSR (C2)		√	√		September
11.	-----do-----	SSR (C3)	√			√	September
12.	-----do-----	SSR (C4)		√		√	September
13.	Swollen radicle detached from seed (SRD)	SRD (D1)	√		√		September
14.	-----do-----	SRD (D2)		√	√		September
15.	-----do-----	SRD (D3)	√			√	September
16.	-----do-----	SRD (D4)		√		√	September

We investigated radicle emergence, the formation of swollen radicles and shoot emergence, seedling recruitment and seedling survival percentage and successful seedling establishment. Observations were made at two months intervals for three years, during growing seasons, because rest of the year the area was covered in snow. The first growing season lasted from July until November, because the seeds were sown in the month of July when seed shedding occurred. The second and third growing seasons lasted from April to November.

Results

The results revealed a wide range of variations in the seedling production of different microsites. The FFMS and SFLR placed outside the canopy with/without litter (A3, A4, B3 and B4 in figure 1) had shoot emergence in 4-7% of the seeds. 18-21% of the seeds had swollen and healthy radicles and the remaining 73-83% of seeds deteriorated after radicle emergence, within the first growing season. Swollen and healthy radicles remained as such on the forest floor and some of them were recovered as seedlings during the second growing season. Only 15% of the seedlings were produced by the seeds and the remaining 85% of seeds lasted in the above mentioned microsites. When FFMS and SFLR were placed under the canopy (A1, A2, B1 and B2 in Figure 1) only 10-12% of the seedlings were recovered from the seeds and the remaining 88-90% were deteriorated, irrespective of litter conditions. Regeneration percentage was close in A1, A2, B1 and B2, under the canopy with/without litter, as well as outside the canopy, without litter. However, the seeds placed outside the canopy, with litter (A3 and B3) produced

18-20% of seedlings, which shows the beneficial effect of litter outside the canopy, in contrast to under the canopy conditions, where litter was observed to have the opposite effect.

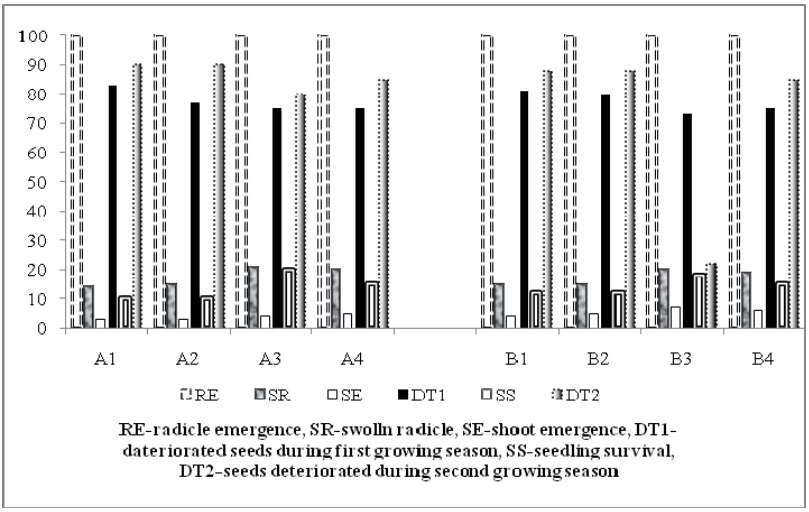

Fig. 1. Regeneration potential of *Q. semecarpifolia* under different microclimatic conditions (A1 to B4, details of the conditions given in the table 1) *in situ*

The findings state that considerably higher number of seeds reached shoot emergence and seedling establishment, when sown after two months of storage (seeds with swollen radicle) when the expansion in radicle diameter had taken place in the approximately 4-5 cm long portion at the middle of the radicle. The seeds with swollen radicles (SSR), when placed under the canopy with/without litter (C1 and C2 in figure 2), reached shoot emergence in 70% of the seeds, during the first growing season**,** 30% of the seeds remained as such over winter and in next growing season, when the snow melted and the temperature of the area increased, we recovered another 20% of seedlings.

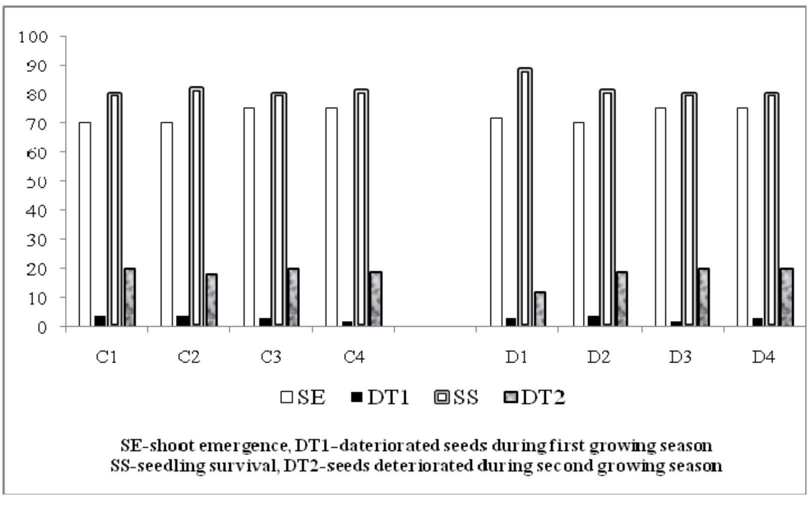

Fig. 2. Regeneration potential of *Q. semecarpifolia* under different microclimatic conditions (C1 to D4, details of the conditions given in the table 1) *in situ*

In total, 80% of the seedlings survived well in those conditions and 20% degenerated during the next growing season. For the SSR placed outside the canopy (C3 and C4), irrespective of litter conditions, shoot emergence occurred earlier and there was a higher percentage of shoot emergence in 75% of the seeds during the first growing season and 5% of the seeds successfully emerged shoot in the next growing season. Only 20% died during winter. During the next growing season, when snow melted in the area, 80% of the seedlings were recovered and that was very satisfactory. Almost similar results were achieved if the seeds were placed outside the canopy (C3 and C4 in figure 2) with or without litter. After the expansion in diameter of the radicle we observed no negative effect of the litter in seedling recruitment.

To examine their tolerance capacity and their ability to act as propagules, detached swollen radicles, before and after expanding in diameter, were used as regeneration propagules. Detached radicles with maximum length, but without expansion in diameter were placed on the forest floor in all the above mentioned microsites. In all the cases the radicles dried/deteriorated in 4-5 days, so there was no data to analyze. Surprisingly, the rest of the radicles remained healthy and viable until the start of next growing season and they produced healthy seedlings in 80-82% (D1, D2, D3 and D4 in figure 2). As regards seedling establishment, it hardly mattered whether the swollen radicles were attached or detached from the seed. The opposite happened when the radicles were detached after attaining their full length (10-12cm), just before the initiation of diameter expansion and then kept in all the microsites described earlier. In all the microsites those radicles dried/deteriorated within 4-5 days.

Discussions

It has long been known that oak forests are mismanaged and that the key to their rejuvenation is successful oak regeneration [9, 18]. Still, the success of oak regeneration in the area we studied is a big challenge, because of the uncontrolled use of resources, grazing, predation of acorns by wild animals, birds and insects, climate changes, insufficient annual seed crop and unpredictable seed behaviour [3, 5, 8, 19].

The present study deals with the following two important factors in the regeneration potential of *Q. semecarpifolia* in its natural habitat: (a) role of different microsites and (b) the selection of proper planting stock.

Regeneration potential of *Q. semecarpifolia* was very high in laboratory conditions [5], but in natural condition the results were not very satisfying. Our findings show that among all the selected microsite conditions, the conditions outside the canopy, with presence/absence of litter (A3, A4 and B3, B4 in figure 1) are the best suited conditions, as 18-20 % seedling establishment occurred in the *Q. semecarpifolia* acorns of freshly fallen seeds (FFMS) and in the seeds stored for one month with full length radicle (SFLR). Contrary to outside the canopy conditions, had an inhibitory effect under the canopy (A1, A2, B1 and B2 in figure 1), where only 8-10% seedlings were recovered. The beneficial effect of litter outside the canopy could be mediated through decreased desiccation and retaining sufficient seed moisture and its 10-15cm long and 0.5 cm thick radicle. That confirms the findings of J. Young and R. Evans [20] who pointed out that litter increased relative humidity at the soil surface by about 30% and delayed depletion of soil moisture at the surface, thus, resulting in increased germination. However litter had adverse effects on seedling emergence under the canopy, as the seeds placed under the canopy with litter had a higher percentage of degenerated seeds. Partial light, very high moisture and microorganisms and/or their metabolic products are also known to influence regeneration. Those factors might have contributed to the higher number of seeds that degenerated under the canopy with litter, as compared to those without litter, as the poor light regime and constant damp conditions encourage the growth of fungi and other microorganisms. The number of seedlings in the oak stands correlated with factors indicating increased disturbances, *i.e.* increasing light intensity and decreasing canopy cover [21]. According to S. Tashi [15] *Q. semecarpifolia* germination does not depend on canopy openness, but it is better with more canopy cover for the initial years of the seedling stage. S. Tashi [15] also suggested that for the oak seedlings to survive there seems to be a need for shade in the initial years, as young plants can tolerate reasonable levels of shade, but during later stages they need more light in order to grow and develop into the next phase, because older trees do not tolerate shade.

A high percentage of seeds degenerated on the forest floor even after radicle emergence, the percentage of such seeds ranging between 80 and 90%, depending on the conditions in which seeds were placed. Similar results were found in *Bouteloua rigidisita* and *Asistida longisita* [22]. E. Gardiner [23] and J. Lhotka and J. Zaczek [24] also concluded that an adequate level of light in the understory is crucial to the development of bottomland oak seedlings and that low light levels limit the adequate development of oak seedlings. pp. Thakuri [8] also reached similar results and noticed that the canopy openness enhances the regeneration, but litter and soil attributes had no significant relations with the density of seedlings and saplings.

In our previous trials [5] only freshly fallen seeds (FFMS) were used under different microsites but very disappointing results were obtained after repetitive experimentation. Although radicle emergence took place in approximately all the seeds, only 20% produced seedlings, and 4% reached to the sapling stage. Thus, to determine the environmental impact under different conditions during seedling establishment and to select proper planting stages/propagules, not only immediately fallen mature seeds but also seeds stored for two months in sterilized sand were used, producing different stages leading to seedling production. The deterioration of radicles caused maximum mortality, due to mildew and other microbe attacks and their sensitivity to desiccation or frost. To avoid the deterioration of radicles in nature it was very challenging to identify the proper planting stock or propagule able to overcome the problem of mildew and desiccation or frost damage. To solve the problem, the seeds were placed on the forest floor when radicles had acquired the ability to tolerate desiccation and frost and became resistant against microbial attacks. We were fortunate enough to identify the proper planting stock/propagule during storage, when seeds grow a radicle with a maximum length, expanding in diameter at the middle. At that stage the swollen radicle was resistant against microorganisms, desiccation and frost and worked well as a planting stock. During two months of storage in sterilized sand, the seeds developed swollen radicles. Keeping the characteristics of swollen radicles in mind, those seeds were used for seedling production and 80-82% of the seedlings were recovered during the second growing season and they survived in all the microsites (C1 to C4 in figure 2) until the end of the third growing season and they rooted well. That indicated the fact that after the development of a swollen radicle it hardly mattered which microsite condition the planting material was sown to regenerate.

It is also remarkable that after the development of a swollen radicle, the rest of the seeds degenerate gradually, which indicates the independence of the swollen radicle from the cotyledons. It is again very interesting to investigate that swollen radicles successfully emerge shoots, even after detachment from the seed, in natural conditions, irrespective of microsites. It is very unusual with this oak species [5] that the swollen radicles detached from the seed and then kept as a propagule germinate under the canopy and outside the canopy with or without litter. Around 70-75% of the radicles reached shoot emergence from the swollen portion, as in the case of the attached swollen radicle. That fact made us investigate the possibility that swollen radicles might survive independently and produce seedlings, even after detachment of the seed and we reached surprising results. We concluded that radicles completely depend on seed cotyledons until the expansion in diameter and can not survive in any condition. Contrary to this, if swollen radicles were detached from the seed after two months of storage and sown in different microsites, 80-82% seedlings were produced (D1 to D4 in figure 2). Those results were similar as in the case of attached swollen radicles with seed, irrespective of the composition of the substratum present in different microsite conditions. It supports the finding that the radicle acquires resistance against unfavorable conditions, such as frost, desiccation and microbial attacks, after expanding in diameter, so it can survive well in unfavorable conditions, even after detachment of the seed. It must be an adaptive strategy of the seeds to survive in the area with high light intensity in the summer and heavy snowfall during winter. Likewise the seeds of some other species (*Quercus, Trillium, Viburnum, Convallaria and Polygonatum)* also exhibit epicotyle dormancy where seeds germinate and develop a radicle in the autumn, without prior frosting of the seed [25]. However the development of the epicotyle depends on a frosting treatment and that does not normally occur during spring. Seedlings of those species do not emerge above the ground before the second spring after seed dispersal [26]. Such phenomena, as acclimatization according to the harsh environmental conditions that exist in nature, are of ecological implication. It indicates that radicles totally depend on seed cotyledons and cannot survive in any condition before the expansion in diameter of the radicle. It was very important to notice that once the seedlings established, towards the end of second growing season, the mortality rate was very low. Almost all the seedlings survived the forthcoming winter and started growing during the third growing season. The most exciting and important part of our study was understanding the importance and ecological implication of the development of swollen radicles, which is a crucial event or phase in regeneration [27].

A. Gallo et al. [28] states that researchers dealing with conservation subjects usually do not put the results of their work into practice, even when the primary purpose of their research is the preservation of biodiversity.

Conclusions

Our findings on regeneration trials and selection of proper planting propagule for the conservation of *Q. semecarpifolia* did not only help understand the regeneration facts, but also contributed to the efforts of forest planners, managers and nursery developers concerned to restore the Himalayan mountains forest structure, as the forest structure is the framework in which the system functions and which restricts and guides the ecosystem. Moreover, it was very interesting to investigate the autonomy of the swollen radicle part of *Q. semecarpifolia* and its regeneration potential.

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