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Original Article

Effects of Pre-sowing Treatments on Seed Germination of Oaks in Kumaun, West Himalaya

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Abstract

The noticeable decline in natural regeneration of three important species of West Himalayan oaks, namely *Quercus glauca*, *Q. leucotrichophora* and *Q. lanuginosa*, on account of excessive lopping, over grazing and tree felling accompanied by non-viable seeds due to short viability, extreme weevil and pest infestation, animal and bird predation resulting in low acorn production and thus overall poor natural regeneration, is of great concern. A study was therefore, carried out with an objective to find out possibilities of improving germination ability of selected oak species using different pre-sowing treatments on three seed size classes- small, medium and large collected from Nainital region of Uttarakhand. A wide variation in seed size existed within these species and germination was found to vary with seed weight; large and medium size seeds exhibiting higher germination. Under various pre-sowing treatments, seeds from different species did not reflect uniformity in responses. Among all the set of experiments, water soaking (48 h) proved to be the best and cost effective approach by significantly improving seed germination over control, *Q. glauca* (53.3 to 73.3%), *Q. leucotrichophora* (66.67 to 90.0%) and *Q. lanuginosa* (58.33 to 75.0%). However, acid scarification emerges as effective pre-sowing treatment. Both water soaking and acid scarification treatments also helped in reducing Mean Germination Time. The outcomes of this study clearly reflect some of the simple, practical and cost effective methods for mass seedling production and restoration of degraded hills in west Himalaya.

Keywords: acorn size; Himalayan oaks; pre-sowing treatments; seed germination; *Quercus glauca*; *Q. leucotrichophora*; *Q. lanuginosa*

Introduction

Oaks (genus *Quercus*) form an important group of trees (family Fagaceae) not only in the Indian sub-continent, but also in Europe, North America, Japan, etc, primarily occurring in temperate to sub-temperate climate. There are over 600 oak species distributed across the world, with maximum diversity in Mexico (around 160 species; 109 as endemics) and China (over 100 species) (Oldfield and Eastwood, 2007). In India are over 35 species of oaks are reported (Negi and Naithani, 1995).

In the west Himalaya, 5 species of evergreen oaks, namely *Q. leucotrichophora* (Banj), *Q. glauca* (Phaliyant), *Q. lanuginosa* (Rianj), *Q. floribunda* (Tilonj/Moru) and *Q. semecarpifolia* (Kharsu) grow naturally. These species are widely distributed, gregarious in occurrence, and well known for their economic and ecosystem values.

However, poor natural regeneration is of great concern for west Himalayan oaks due to several reasons, like, excessive lopping, over grazing and tree felling, non-viable seeds, extreme weevil and pest infestation, animal and bird predation resulting in low acorn production (Saxena and Singh, 1984; Thadani and Ashton, 1995). Seed germination, seedling performance, natural regeneration and subsequent growth in oaks are often attributed to their recalcitrant nature and wide variation in acorn size within a species (Tripathi and Khan, 1990; Khan and Shankar, 2001; Purohit *et al*., 2003; Tilki and Alptekin, 2006).

In general, efforts of clonal propagation (Bhardwaj *et al*., 1996; Tamta *et al*., 2000) have not much succeeded in most oak species in the region. Therefore, improvement of natural regeneration and mass propagation using seeds seems to be most viable alternative.

The present study, therefore, is an attempt to improve seed germination, using various pre-treatments, in three oak species (i.e., *Q. leucotrichophora*, *Q. glauca* and *Q. lanuginosa*) of the region. The study intends to identify best responding treatments so as to promote the large scale production of planting material for these highly preferred species in west Himalaya.

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Materials and Methods

Seed collection

Mature seeds (acorns) of all three target species were collected from different forest sites near Nainital (Kumaun forest division, Uttarakhand). Following the seed maturation, seeds of *Q. glauca* were collected in the month of October/November, *Q. leucotrichophora* in November/December and *Q. lanuginosa* in December/January (year 2014-2015). Geo-coordinates and altitude of each site was recorded using hand held Global Positioning System [GPS (Garmin make)]. Seeds after measuring fresh weight were stored in a paper bag at temperature (25 ± 2 °C) until the experimentation.

Morphological analysis

A total of 30 seeds (10 seeds × 3 sets = 30) per species were randomly selected and various morphological attributes like acorn length, acorn width and acorn fresh weight were recorded. Acorn length and width were measured using digital Vernier's calliper (Mitutoya, Japan) and fresh weight using weighting balance (Citizen Scale CY 510).

Determination of weight classes

Randomly selected 100 seeds for each of the target species were weighted for the fresh weight. Considering minimum and maximum fresh weight values, ranging from 1.18 g to 2.42 g for *Q. glauca*, 1.15 g to 2.61 g for *Q. leucotrichophora* and 0.89 g to 1.95 g for *Q. lanuginose*, three size classes (small, medium and large) were made (Table 1).

Determination of moisture content

Seed moisture content was determined using ten seeds for each of the three replicates. After taking fresh weight, seeds were oven dried at 60 °C for 48 h. The oven dried seeds were reweighted and the moisture content was calculated as: Moisture (%) = FW-DW×100/FW, where FW is Fresh weight (g) and DW is weight after oven drying (g).

Viability test

Viability of seeds across weight classes of each species was assessed following the standard approach (Hendry and Grime, 1993). Thirty seeds (three replicates, each with ten seeds) from each weight class were immersed in a 1% aqueous solution of 1, 2, and 3- triphenyl tetrazolium chloride (pH- 6.0) for 24 h in dark (25 ± 2 ° C). Percent viability was determined by calculating the number of stained embryos in each weight class.

Seed size and germination responses

Germination across weight classes of untreated seeds (three sets of 10 seeds each) was performed on selected oak species. This germination test on untreated seeds served as control for respective weight class of given species. Seeds were placed in Petri plates (95 x 17 mm) containing moistened filter paper (Whatmann No. 1). Petri plates were kept in growth chamber at a constant temperature (25 ± 2 °C). Seeds were considered germinated when the tip of the radical emerged (2 mm) from the seed. Thereafter, weekly observations were recorded. Mean Germination Time (MGT) was calculated as: MGT= $\sum$ (n × d)/N; where n= the number of seeds which germinated after each period of incubation in days d and N = the total number of seeds emerged at the end of the test (Hartmann and Kester, 1989).

Pre-sowing treatments and germination responses

Based on the preliminary results it was noticed that medium and large size seeds in all three species germinate more with lesser Mean Germination Time. Therefore, pre-sowing treatments were applied on pooled seed lots of medium and large weight class. Seeds were pre-treated with Bavistin (0.05%) solution for 30 minutes, washed 3-4 times thoroughly with distilled water and placed in beakers containing 100 ml of various test solutions for 48 hours. The following tests were conducted for detailed investigation.

Water soaking treatment

Seeds were soaked in distilled water (12, 24, 36 and 48 hours); water changed after every 12 h (Hartmann and Kester, 1989). The soaked seeds were incubated on Petri-plates and observations were recorded. Treated seeds were subsequently subjected to germination test.

Acid scarification

Acid scarification was performed by placing seeds in beakers containing sulphuric acid (conc. H_2SO_4) for 5, 10 and 20 min and occasionally shaken. Immediately after treatment, seeds were washed 3-4 times vigorously under tap water. Treated seeds were allowed to imbibe for 48 hours in distilled water before incubating on Petri-plates for germination.

Plant Growth Regulators (PGRs) and chemical treatments

Seeds were soaked for 48 hours to examine the effect of different concentration of plant growth regulators [Gibberellic acid (GA_3) and Indole-3- acetic acid (IAA)]; 100, 200 and 400 μM) and nitrogenous compounds (Thiourea and KNO_3; 50 mM and 100 mM) on germination.

Table 1. Seed size class distribution in three oak species

	Fresh weight (gm)		
Weight class→ Species↓	Small	Medium	Large
Q. glauca	< 1.50	1.50-2.00	> 2.00
Q. leucotrichophora	< 1.50	1.50-2.00	> 2.00
Q. lanuginosa	< 1.00	1.00-1.50	> 1.50

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and multivariate analysis using SPSS programme (version 16.0) for comparison of the means of germination percentage on different treatments. The significance level was determined at $p < 0.05$ and the means were separated using Duncan's multiple range test (DMRT), if the values were significantly different. Data are presented as mean values ± standard error (SE).

Results

Major attributes on seed morphology, moisture content and viability

Variations in all morphological characteristics of acorns in the targeted species were observed and recorded (Table 2). Among the studied oaks, *Q. leucotrichophora* was found to have maximum seed weight (1.15-2.61 g), seed length (20.81 mm) and width (11.66 mm). Seed size was found minimum for *Q. lanuginosa*.

In all the species, moisture content increased with increasing seed size (*Q. glauca* 15.2-23.3%; *Q. leucotrichophora* 16.5-28.5%; *Q. lanuginosa* 10.3-19.2%). This increase of moisture content was significant ($p < 0.05$) in all the species. Similarly, the viability also increased with the size of seeds (*Q. glauca* 53.3-66.6%; *Q. leucotrichophora* 46.7-90.0%; *Q. lanuginosa* 43.3-56.7%). However, the increase was significant ($p < 0.05$) only in the case of *Q. leucotrichophora* from small to medium and large category seeds.

Effect of seed size on germination responses

Percentage germination was found to increase with increasing seed size from 46.67% to 53.33% for *Q. glauca*, 46.67% to 70% for *Q. leucotrichophora* and 43.33% to 60% for *Q. lanuginosa*. With increasing seed size significant ($p < 0.05$) reduction was noticed in MGT except for *Q. lanuginosa* (Table 3).

Effect of pre-sowing treatments on germination

Germination responses across different pre-sowing treatments varied significantly (Table 4). Germination responses under different water soaking treatments revealed that the germination increased with increasing soaking duration up to 48 h, which was significant over control ($p < 0.05$) in all the species. The highest mean germination (90%) under 48 h water soaking treatment for *Q. leucotrichophora* was considerably better than the other two species (*Q. glauca*-73.31%; *Q. lanuginosa*-75%). This treatment also significantly ($p < 0.05$) reduced the MGT over control in *Q. glauca* (96.88 d to 78.35 d), *Q. leucotrichophora* (95.39 d to 69.68 d) and *Q. lanuginosa* (71.63 d to 60.87 d).

As compared to control, sulphuric acid scarification treatments helped improving germination. Germination percentage increased with increase in treatment duration up to 10 min from 53.33% (control) to 75% in *Q. glauca* and 58.33% (control) to 78.33% in *Q. lanuginosa*. Further increase in time duration in general lowered the mean germination [except in *Q. leucotrichophora* where further increase in germination (83.33%) was observed under 20 min treatment]. Acid scarification treatment invariably reduced the MGT and reduction was significant over control in most cases.

Among PGRs, IAA did not prove effective in improving germination in all the studied species. Whereas, seeds soaked in GA_3 showed significant increase ($p < 0.05$) in germination percentage with increasing concentration up to 400 µM from 53.33% (control) to 73.33% in *Q. glauca* and 58.33% (control) to 68.33% in *Q. lanuginosa* [except for *Q. leucotrichophora* where the mean germination (81.67%) was higher for 200 µM of GA_3]. Considering MGT, the increased concentration of GA_3 mostly helped in reduction in MGT except for *Q. lanuginosa* where reduction in MGT was non-significant ($p > 0.05$).

Table 2. Major seed characteristics of three oak species in Kumaun, West Himalaya

Attributes	*Quercus glauca*	*Quercus leucotrichophora*	*Quercus lanuginosa*
Seed collection site	Kalona (Nainital)	Maheshkhan (Nainital)	Kilbury (Nainital)
Altitude (m asl)	1,470	1,950	2,230
Geographical co-ordinates	N 24°20′ E 79°27′	N 29°24′ E 79°32′	N 29°25′ E 79°26′
Seed maturation time	October - November	December - January	January
Seed coat	Hard	Hard	Hard
Mean seed length (mm)	19.92 ± 0.36	20.81 ± 0.18	17.66 ± 0.10
Mean seed width (mm)	9.40 ± 0.04	11.66 ± 0.06	9.47 ± 0.14

Table 3. Effects of seed size on mean germination and MGT in three oak species in Kumaun, West Himalaya

Weight class	QG	QL	QLG	QG	QL	QLG
	Germination responses (%)			MGT (d)		
Small	46.67 ± 3.34^{a}	46.67 ± 6.67^{a}	43.33 ± 8.83^{a}	111.07 ± 4.98^{a}	97.80 ± 2.48^{ab}	78.00 ± 7.98^{a}
Medium	53.33 ± 3.34^{a}	63.33 ± 3.3^{a}	56.67 ± 8.83^{a}	99.63 ± 2.40^{ab}	102.27 ± 5.42^{a}	75.83 ± 10.36^{a}
Large	53.33 ± 3.34^{a}	70.00 ± 10.01^{a}	60.00 ± 5.78^{a}	94.14 ± 4.25^{b}	88.51 ± 2.68^{b}	67.43 ± 10.11^{a}

Values are mean ± standard error; different super script letters in a column indicate significant variation ($p < 0.05$) based on Duncan multiple range test (DMRT) (QG = *Q. glauca*; QL = *Q. leucotrichophora* and QLG = *Q. lanuginosa*)

Responses of seeds immersed in nitrogenous compounds were more or less similar to GA_3 treated seeds. In case of chemical treatments, KNO_3 (100 mM) was found better for improving germination from 66.67% (control) to 85% followed by Thiourea (50 mM) to 80% in *Q. leucotrichophora* than other two species. Compared to control, except for *Q. lanuginosa*, these treatments were also found effective in reducing MGT significantly ($p < 0.05$).

Table 4. Germination responses and MGT under various pre-sowing treatments in three oak species in Kumaun, West Himalaya

Pre-treatment	Conc./Duration	QG	QL	QLG	QG	QL	QLG
		Germination response (%)			MGT (d)		
Control	-	53.33 ± 2.12^{g}	66.67 ± 4.96^{bc}	58.33 ± 4.79^{cdef}	96.88 ± 2.51^{ab}	95.39 ± 4.11^{a}	71.63 ± 6.76^{a}
GA_3	100 µM	56.67 ± 2.12^{efg}	73.33 ± 8.06^{abc}	60.00 ± 2.59^{cdef}	91.38 ± 1.74^{bc}	75.15 ± 2.83^{cde}	69.34 ± 3.65^{a}
	200 µM	60.00 ± 2.59^{defg}	81.67 ± 3.09^{ab}	65.00 ± 2.24^{bcd}	90.89 ± 2.42^{bc}	71.06 ± 2.36^{def}	69.31± 2.31^{a}
	400 µM	73.33 ± 3.35^{ab}	65.00 ± 7.67^{bc}	68.33 ± 4.03^{abc}	76.22 ± 1.51^{gh}	66.90 ± 2.66^{efg}	68.67± 1.61^{a}
IAA	100 µM	53.33 ± 4.23^{g}	68.33 ± 6.03^{bc}	50.00 ± 4.49^{f}	100.04 ± 2.62^{a}	91.80 ± 2.67^{a}	70.27± 3.41^{a}
	200 µM	55.00 ± 2.24^{fg}	60.00 ± 6.86^{c}	51.67 ± 4.03^{ef}	97.84 ± 2.08^{a}	81.91± 1.70^{bc}	70.42 ± 2.66^{a}
	400 µM	56.67 ± 2.12^{efg}	65.00 ± 6.22^{bc}	53.33 ± 4.23^{def}	96.18 ± 2.26^{ab}	88.27 ± 4.85^{ab}	69.99 ± 3.33^{a}
KNO_3	50 mM	63.33 ± 3.35^{bcdefg}	73.33 ± 4.23^{abc}	75.00 ± 4.30^{ab}	85.13 ± 1.61^{cde}	64.47 ± 2.37^{fgh}	66.51± 2.22^{ab}
	100 mM	58.33 ± 3.09^{efg}	85.00 ± 4.30^{ab}	66.67 ± 2.12^{abc}	83.67 ± 1.26^{def}	69.50 ± 2.29^{defg}	64.55 ± 2.46^{abc}
Thiourea	50 mM	63.33 ± 2.12^{bcdefg}	80.00 ± 5.18^{abc}	58.33 ± 4.79^{cdef}	82.62 ± 1.87^{ef}	62.13 ± 1.42^{fgh}	66.27±1.91^{ab}
	100 mM	70.00 ± 3.67^{abcd}	73.33 ± 8.85^{abc}	60.00 ± 3.67^{cdef}	77.75 ± 1.50^{fgh}	62.98 ± 4.12^{fgh}	66.71± 4.10^{ab}
Water soaking	12 h	61.67 ± 3.09^{cdefg}	68.33 ± 7.95^{bc}	63.33 ± 4.23^{bcde}	99.87 ± 2.06^{a}	76.69 ± 2.11^{cd}	66.09 ± 2.64^{ab}
	24 h	65.00 ± 2.24^{abcdef}	80.00 ± 3.67^{abc}	65.00 ± 4.30^{bcd}	89.82 ± 2.39^{cd}	67.68 ± 3.20^{efg}	66.12 ± 2.66^{ab}
	36 h	66.67 ± 3.35^{abcde}	85.00 ± 3.43^{ab}	66.67 ± 4.23^{abc}	85.27 ± 2.11^{cde}	68.43 ± 1.55^{defg}	65.04 ± 3.81^{abc}
	48 h	73.33 ± 3.35^{ab}	90.00 ± 4.49^{a}	75.00 ± 4.30^{ab}	78.35 ± 2.68^{fgh}	69.68 ± 3.34^{defg}	60.87 ± 3.19^{abc}
Acid scarification	5 min	71.67 ± 3.09^{abc}	78.33 ± 6.03^{abc}	75.00 ± 4.30^{ab}	82.33 ± 2.14^{efg}	61.97 ± 1.72^{fgh}	57.87± 0.63^{bc}
	10 min	75.00 ± 3.43^{a}	75.00 ± 6.22^{abc}	78.33 ± 3.09^{a}	73.76 ± 1.63^{h}	60.53 ± 2.42^{gh}	55.58 ± 2.54^{c}
	20 min	53.33 ± 5.60^{g}	83.33 ± 5.60^{ab}	56.67 ± 4.23^{cdef}	87.61 ± 1.38^{cde}	57.43 ± 1.03^{h}	64.63 ± 2.00^{abc}

Values are mean ± standard error; different super script letters in a column indicate significant variation ($p<0.05$) based on Duncan multiple range test (DMRT) (QG= *Q. glauca*; QL= *Q. leucotrichophora* and QLG= *Q. lanuginosa*)

Discussion

The study provides useful understanding on possible improvement of germination responses in targeted three Himalayan oak species. The improved seed germination and decreased MGT with increasing seed weight is in general in agreement with earlier reports for some other oaks, which have often attributed these responses to high energy and nutrient reserves of larger seeds (Tripathi and Khan, 1990; Bonfil, 1998; Bhuyan *et al*., 2000; Khan and Shankar, 2001).

Positive response of seeds of selected oak species for water soaking supports the findings of Purohit *et al.* (2009), which reports 48 h of water soaking duration as most effective for achieving highest germination in *Q. glauca* and *Q. leucotrichophora*. The better response with increasing soaking duration suggests that the oak seeds require an optimal level of moisture to activate the embryo to commence the process of cell division, differentiation and multiplication to grow into a seedling. Water soaking has also been found effective for seeds of *Q. laurifolia* (Larsen, 1963) and other important wild tree species in the region, for example *Cornus capitata* (Airi *et al*., 2005) and *Myrica esculenta* (Bhatt *et al*., 2000).

Improved germination under acid scarification can be attributed to the fact that sulphuric acid breaks physical (seed coat) dormancy by corroding the outermost layers of the seed coat, improving seed permeability and thereby enhance germination (Ren and Tao, 2004; Murat *et al*., 2010). The present study, therefore, supports that acid scarification is simple and easy to apply on a large lot of seeds over other tedious, labour intensive and time consuming methods of breaking physical dormancy.

Gibberellic acid was found to enhance germination percentage with reduction in MGT in the present study. GA_3 is known to promote seed germination by releasing dormancy (Nickell, 1982), mobilize nutrients (Kumar and Purohit, 1986), rupture pericarp by cotyledonary expansion and thereby improve germination (Bradbeer, 1988). Significant improvement in germination of *Q. leucotrichophora* seeds under GA_3 (200 µM) treatment corresponds with similar reports in case of *Q. rubra* (Vogth, 1970) and *Q. falcate* (Bonner, 1976).

Although the beneficial effect of IAA has also been reported (Chatterjee, 1960), however, treatments with varying concentrations of IAA were found either ineffective or inhibitory in the present study. It may be due to the reason that IAA can stimulate transcription of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase. This enzyme facilitates a key step in ACC oxidase-mediated ethylene biosynthesis (Mayak *et al*., 1999). The IAA-derived ethylene is believed to participate in the disruption of the normal growth (*i.e*. germination and seedling growth) of the host plant.

Among nitrogenous compounds, use of Thiourea and KNO_3 on oak seeds remained less effective. Thiourea has been reported by others to break seed dormancy and improve germination (Agarwal and Dadlani, 1995; Pandey *et al*., 2000). Likewise, Purohit *et al.* (2009) reported KNO_3 treatments to be highly effective in improving seed germination of *Q. glauca* and *Q. leucotrichophora*. Present study, however, does not fall in full agreement with these reports.

Conclusions

The study concludes the following: (i) larger size seeds of studied oaks respond better for germination, (ii) water soaking (48 h) is most effective treatment for enhancing germination, (iii) acid scarification also emerges as suitable pre-sowing treatment. While considering the cost effectiveness, water soaking seems to be the most potential treatment for being accepted by rural communities towards promotion of community forestry and non-government organization for nursery development to support large scale plantations in the region.

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