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Effect of pre-sowing treatments on seed germination in *Quercus serrata* Thunb. and *Quercus semecarpifolia* Sm.

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Quercus **species are major forest forming species and helps in sustaining an ecosystem. Natural regeneration of these species is very poor due to various biological and physical factors. The present study was conducted to evaluate the effect of different pre-sowing treatments (scarification and cold-stratification) on seed germination of two *Quercus* species, that is, *Quercus serrata* (deciduous) and *Quercus semecarpifolia* (evergreen). On the basis of the present study, it was observed that pretreated seeds germinated better in comparison with control, and both species responded differently to the treatments. The deciduous, lower altitude *Q. serrata* gave better results in comparison with the evergreen, high altitude *Q. semecarpifolia* in terms of percent germination and time taken for germination. Being a simple and cost effective method, the results of the present study could be used for developing nurseries for commercial purposes.**

Key words: Pre sowing treatments, seed germination, stratification, scarification, *Quercus*.

INTRODUCTION

Seeds provide the most natural means of plant reproduction, preservation of genetic variability, transportation and propagation in angiospermic plants (Vazquez and Rojas, 1996). Propagation through seeds is considered to be one of the most reliable, efficient and universally applied methods (Hartmann and Kester, 1990).

Oaks are naturally propagated through seeds, but regeneration through seeds in nature is poor (Troup, 1921). In many cases, viable seeds do not germinate even under favorable environmental conditions; this phenomenon is termed seed dormancy (Taiz and Zeieger, 2002). Several internal factors cause dormancy which include seed coat, embryo or inhibitors which influence the seed germination rate (Agrawal and Dadlani, 1995). Different methods such as heating (Herranz et al., 1999), stratification, scarification (Narbona et al., 2003) and gibberellin application are well known to overcome these problems depending on the type of plant species and dormancy. Moist chilling or cold stratification is widely used for breaking seed dormancy and increasing the rate of germination percentage of dormant seeds of many species (Wang and Berjak, 2000). Influence of various concentrations of growth substances in seed germination and seedling growth was reported by Dhoran and Gudadhe (2012). However, the responses are species specific and no one treatment has been reported to be effective universally (Clemens et al., 1977).

Since the scarification and cold-stratification treatment are less expensive methods for seed germination with promising outcomes, these were tested to improve seed germination rate and establish a cost-effective method for enhancing the germination rate of two *Quercus* species

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Plate 1. Effect of pre-sowing treatments on seed germination in *Q. serrata* and *Q. semecarpifolia*. a and c: Viability test of seeds by soaking in water, arrow indicate viability difference in both species b: Unscarified and scarified seeds; d-e: comparison of seed germination rate between *Q. semecarpifolia* and *Q. serrata* after 30 days of inoculation.

viz. *Quercus serrata* and *Quercus semecarpifolia.*

MATERIALS AND METHODS

Seed collection

Mature seeds (acorn) of *Q. serrata* were collected (handpicked) from Bhimtal (1,370m asl) and Pithoragarh (1,635m asl) area of Kumaun region, while seeds of *Q. semecarpifolia* were collected from Kunja-khark and Chinapeak area of Nainital (2,619m asl), Kumaun region and Srinagar (2,680m asl) area of Garhwal region, Uttarakhand, India. Acorns of *Q. serrata* and *Q. semecarpifolia* were collected during November-December and July-August 2009, respectively.

Seeds were removed from inflorescence, seeds of *Q. serrata* were dried at room temperature (24±5°C) and stored in airtight polybags at room temperature until use while, seeds of *Q. semecarpifolia* were used as soon as possible because of low viability.

Test for viability

After seed collection, the seed viability was checked by dipping seeds in a container containing tap water (frequently used method for acorns). Seeds having embryo settled down and embryoless seeds floated in the water. Settled down seeds were considered as viable and selected for the present study (Plate 1a and c).

Pre sowing treatments

Scarification

Seed coat was removed with the help of forceps, needle and scalpel. The seed without seed coat served as scarified seed and the seed with seed coat called un-scarified seed served as control (Plate 1b).

Cold-stratification

Both scarified and un-scarified seeds were kept at 4 ± 2°C for 5, 10, 20 and 30 days for cold- stratification.

Laboratory conditions and maintenance

Before giving cold-stratification treatment, viable seeds (scarified as well as un-scarified) were treated with 1% bavistin for 20 min, rinsed with distilled water. After 5, 10, 20 and 30 days interval seeds were taken from the refrigerator and placed in sterile petri dishes (17 x 95 mm) containing moistened filter paper and incubited at room temperature (24 ± 2°C). Humidity was maintained by adding distilled water over filter paper in one day interval. Seeds having emerged radicle were considered germinated. The whole experiment was monitored upto two months.

Mean germination time was calculated according to the method described by Hartman and Kester (1990) by using the following formula:

$$\text{Mean germination time} = \Sigma(n \times d) \div N$$

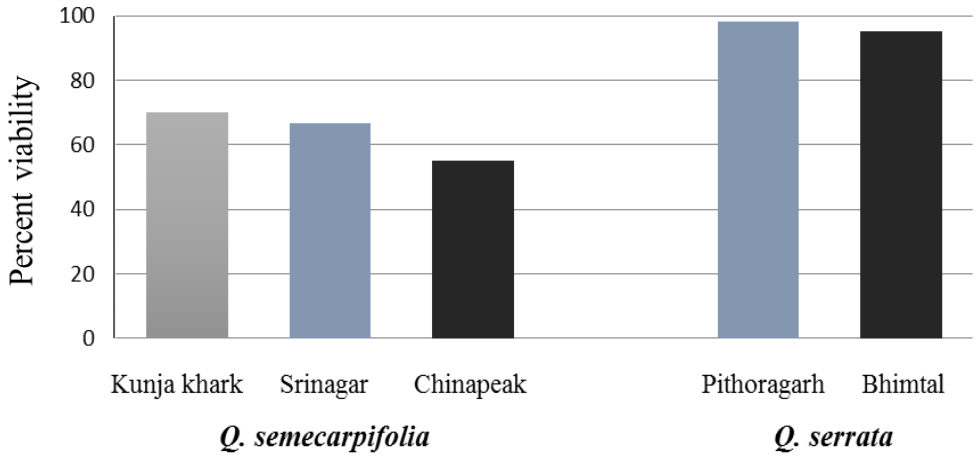

Figure 1. Percent viability of seeds of target species collected from different sites.

Where, n = number of seeds that germinated at time d; N = total number of seeds that germinated; d = days from the beginning of the germination.

Statistical analysis

All experiments were performed in replicates. Three replicates of 6 seeds were analyzed in each treatment. Mean values of treatments were subjected to one way analysis of variance (ANOVA) with the help of SYSTAT (Wilkinson, 1986).

RESULTS

Viability

Viability results showed difference in viability among the target species **(**Figure 1); seeds of *Q. serrata* collected from Pithoragarh showed higher viability (98.33%) than those collected from Bhimtal (95% viability). This may be due to the seed-size, because seeds which were collected from Pithoragarh were large in comparison with seeds collected from Bhimtal (data not shown). *Q. semecarpifolia* seeds collected from Kunja-khark showed maximum viability (70%) whereas seeds collected from China peak possessed least viability (only 55%) (Figure 1). It was observed that percent viability was influenced by seed size, as the larger seeds showed greater percentage of viability in comparison with smaller seeds (detail data not presented).

Pre sowing treatments

Quercus serrata

The results of present study revealed that all the pretreatments significantly increased the average percentage of seed germination when compared with un-scarified and un-stratified seeds (control) (Table 1). In the control no significant effect on germination was observed. Among the treatments, scarification with 20 days stratification was found to be the most effective treatment for breaking the dormancy of *Q. serrata* having 88.67 ± 11.33 percent germination in average time of 11.33 ± 0.67 days as compared to 14.67 ± 3.67 percent germination in average time of 27.67 ± 1.45 days in untreated seeds (control) (Table 1).

Scarification also improved the percent germination rate up to 55.00% and stratification improved germination up to 66.67% when applied for 20 days. Further increase in stratification time decreased the germination rate (44.00%) (Table 1). The results showed that scarification and stratification treatments when applied separately had low effect on the germination rate but scarification with stratification for 20 days at 4 ± 2°C had significantly increased the rate of seed germination and was considered the best treatment. Plate 1e shows germination after 30 days of incubation.

Quercus semecarpifolia

The results of present study showed that pretreatments significantly enhanced the average seed germination rate in comparison to control. Among the treatments, scarification with 10 days stratification was as the found most effective treatment for breaking the dormancy in *Q. semecarpifolia* having 69.67% germination in average time of 12.67 ± 3.18 days as compared with the untreated seeds (control) (33.33% germination in 25 ± 2.89 days) (Table 1). Stratification alone improved germination up to 51.33% when applied for 10 days. Further increase in stratification time decreased the germination rate up to 25.67%. The results showed that scarification had comparatively low effect in the germination rate than stratification and scarification with stratification treatments. Due to high moisture content, after certain point of stratification the germination decreased drastically, was less than control and lost viability. Plate 1d shows germination after 30 days of incubation.

Table 1a. Effect of pre-sowing treatments on seed germination in *Q. serrata and Q.semecarpifolia*.

Treatment (time in days)	*Q. serrata*		*Q. semecarpifolia*	
	Germination (%)	Average germination time (days)	Germination (%)	Average germination time (days)
Scarified	55.00 ±6.35	16.67±2.40	44.44±6.35	21.67±4.41
Stratified (5)	25.67±7.33	21.00±3.79	47.67±3.67	25.00±2.89
Stratified (10)	44.44±11.11	19.00±2.08	51.33±9.70	19.00±3.06
Stratified (20)	66.67±12.70	18.00±2.00	36.67±3.67	20.83±2.85
Stratified (30)	44.00±12.70	18.67±0.88	25.67±3.67	19.67±1.76
Scarified+ Stratified (5)	44.44±6.35	16.67±2.40	62.33±3.67	14.67±2.40
Scarified+ Stratified (10)	69.67±9.70	15.00±0.58	69.67±3.67	12.67±3.18
Scarified+ Stratified (20)	88.67±11.33	11.33±0.67	40.33±3.67	14.00±2.31
Scarified+ Stratified (30)	77.33±11.33	14.00±1.15	29.33±3.67	17.67±0.88
Control	14.67±3.67	27.67±1.45	33.33±12.70	25.00±2.89
LSD (p=0.05)	0.0002*	0.0013*	0.0022*	0.0528^{ns}

Table 1b. Anova.

Source of variation	df	*Q. serrata*				*Q. semecarpifolia*			
		Germination (%)		Average germination time (days)		Germination (%)		Average germination time (days)	
		Mean Square	F-ratio	Mean Square	F-ratio	Mean Square	F-ratio	Mean Square	F-ratio
Between Groups	9	1743.041	6.638	58.82963	4.999	772.61	9.12	55.67	2.36
Within Groups	20	262.6		11.76667		84.7		23.6	
Total	29								

Values represent means ± standard error *significant at 0.5 level, ^{ns}not significant at 0.05 level, Str.- Stratification, twelve explants were used per treatment, the experiments were repeated thrice, and data scored after 4-week.

DISCUSSION

The seed coat is very much hard and thick in *Q. serrata* and may inhibit the rate of seed germination. During this period, it comes in contact with various insects and wild animals which also inhibits the germination, but in the case of internal dormancy stratification is necessary to overcome it. The results of present study showed that scarification (total removal of seed coat) along with stratification significantly improved the germination rate of target species. In *Q. serrata*, scarification contribute to improving the regeneration like other Himalayan oaks viz. *Q. glauca* and *Q. leucotrichophora* (Rawat et al. 1998) where pericarp resisted cotyledon expansion and water uptake of seed during radicle emergence.

Abscisic acid has also been reported as control agent of dormancy (Tillberg, 1983), as stratification proceeds, abscisic acid decreases and giberellic acid is synthesized or released from bound forms (Diaz and Martin, 1972). Giberellic acid accelerates germination by inducing the production of hydrolytic enzymes (Mozer, 1980) and cell plasticity (Taylor and Cosgrove, 1989). It is probably because the scarified seeds when subjected to stratification, the level of giberellic acid increases with the increase of stratification and leads to germination enhancements because gibberellic acid activates the reserve food mobilizing system (Hartman and Kester 1990) while in the control hard seed coat prevents this process and causes germination hindrance of the embryo caused by inability to absorb either water or oxygen.

Some earlier workers viz. Vetaas (2000), Shrestha (2003), Singh and Rawat (2012) and Bisht et al. (2012) also studied the regeneration status of *Q. semecarpifolia* and found very low natural regeneration rate under different microclimatic conditions. Seed germination process in *Q. semecarpifolia* completes in several steps viz. emergence of radicle followed by thickening of radicle and swelling of approximately 2 cm part of radicle takes place, from this swollen portion emergence of root and shoot occur. Bisht (2001) presumed that swollen portion of radicle requires chilling treatment for seedling emergence while Bisht et al. (2012) observed that light has significant effect on seed germination. In *Q. ilex,* Ghasemi and Khosh-Khui (2007) observed that stratification of

scarified seeds positively affected germination rate. In *Q. semecarpifolia,* seeds germinated best in 10 days stratification but as duration of stratification increases the germination rate deteriorated. Bisht (2001) reported that at/below 0°C seeds of *Q. semecarpifolia* lose their viability due to the formation of ice crystal in the cells but seed germination of *Jasminus fruticans* was enhanced by the application of three month cold stratification (Pipinis et al., 2009). Scarification and stratification treatments were able to promote germination and growth of seedling in many of the angiosperms and gymnosperms (Esen et al., 2007), not only in herbaceous but also in tree species like *Solanum nigrum* (Suthar et al., 2009), *Prunus scoparia* and *Prunus webbii* (Heidari et al., 2008) and *Aeluropus lagopoides* (Zaman et al., 2011).

Conclusion

Q. serrata responded considerably better than the *Q. semecarpifolia* in applied germination enhancement experiment. Although, some seeds of *Q. semecarpifolia* in control failed to develop shoot and root even after emergence of radicle and died but it was found that stratification along with scarification improved germination rate significantly in both the target species in comparison with individual stratification and scarification treatments. The present study was able to enhance the seed germination rate and improve germination time. Being a simple and cost effective method, the results of the present study could be used for developing nurseries for commercial purposes.

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