

Full Length Research Paper

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

Aseesh Pandey and Sushma Tamta\*

Plant Tissue Culture and Molecular Biology Laboratory, Department of Biotechnology, Bhimtal Campus, Kumaun University, Nainital-263136, Uttarakhand, India.

Accepted 21 August, 2013

*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

\*Corresponding author. E-mail: sushmatamta@gmail.com



**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

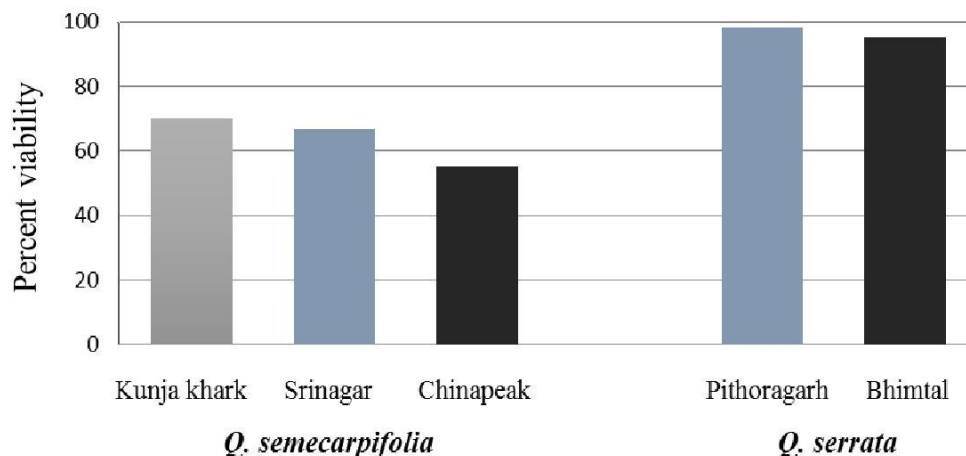
Both scarified and un-scarified seeds were kept at 4 ± 2°C for 5, 10, 20 and 30 days for cold- stratification.

#### 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 incubated 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:

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



**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  $88.67 \pm 11.33$  percent germination in average time of  $11.33 \pm 0.67$  days as compared to  $14.67 \pm 3.67$  percent germination in average time of  $27.67 \pm 1.45$  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  $4 \pm 2^\circ\text{C}$  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  $12.67 \pm 3.18$  days as compared with the untreated seeds (control) (33.33% germination in  $25 \pm 2.89$  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)	<i>Q. serrata</i>		<i>Q. semecarpifolia</i>	
	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 <sup>ns</sup>

**Table 1b.** Anova.

Source of variation	df	<i>Q. serrata</i>				<i>Q. semecarpifolia</i>			
		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, <sup>ns</sup> 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 gibberellic acid is synthesized or released from bound forms (Diaz and Martin, 1972). Gibberellic acid accelerates germination by inducing the production of hydrolytic enzymes (Mozar, 1980) and cell plasticity (Taylor and Cosgrove, 1989). It is probably

because the scarified seeds when subjected to stratification, the level of gibberellic 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.

## ACKNOWLEDGEMENTS

Authors are thankful to GBPIHED Kosi-Katarmal Almora for financial support and head, Department of Botany, D.S.B. Campus Kumaun University Nainital for providing necessary facilities.

## REFERENCES

- Agrawal PK, Dadlani M (1995). Techniques in Seed Science and Technology, 2nd Edn., South Asian Publishers, New Delhi, pp.109-113.
- Bisht H (2001). Physiobiochemical aspects of seed viability in *Quercus semecarpifolia* smith: A possible recalcitrant seed. Ph.D. Thesis, H.N.B. Garhwal University, Srinagar (Garhwal), 246 174, Uttarakhand, India, pp.45-48.
- Bisht H, Prakash V, Nautiyal AR (2012). Factors Affecting Regeneration Potential of *Quercus semecarpifolia*, Smith: A Poor Regenerated Oak of Himalayan Timberline. Res. J. Seed. Sci. 5:63-70. DOI: 10.3923/rjss.2012.63.70.
- Clemens J, Jones PG, Gilbert NH (1977). Effect of seed treatments on germination in *Acacia*. Aust. J. Bot. 25:269–276.
- Dhoran VS, Gudadhe SP (2012). Effect of Plant Growth Regulators on Seed Germination and Seedling Vigour in *Asparagus sprengeri* Regel. I. Res. J. Bio. Sci. 1(7):6-10.
- Diaz DH, Martin GC (1972). Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. J. Am. Soc. Hortic. Sci. 97:651-654.
- Esen D, Yildiz O, Sarginci M, Isik K (2007). Effects of different pretreatments on germination of *Prunus serotina* seed sources. J. Environ. Biol. 28:99-104.
- Ghasemi M, Khosh-Khui M (2007). Effect of stratification and growth regulators on seed germination and seedling growth of *Quercus ilex* L. J. Plant Sci. 2:341-346.
- Hartmann HT, Kester DE (1990). *Plant Propagation*, Englewood Cliffs, NJ: Prentice-Hall.
- Heidari M, Rahemi M, Daneshvar MH (2008). Effect of mechanical, chemical scarification and stratification on seed germination of *Prunus scoparia* (Spach.) and *Prunus webbii* (Spach.) Vierh. Am.-Eurasian J. Agric. Environ. Sci. 3:114-117.
- Herranz JM, Ferrandis P, Martínez Sánchez JJ (1999). Influence of Heat on Seed Germination of Nine Woody *Cistaceae* Species. Int. J. Wildl. Fire, 9:173-182.
- Mozer TJ (1980). Control of protein synthesis in barley aleurone layers by the plant hormones gibberellic acid and abscisic acid. Cell, 20:479-485.
- Narbona E, Arista M, Ortiz PL (2003). Seed germination of *Arbutus unedo* L. (*Ericaceae*). Acta Bot. Mala, 28:73–78.
- Pipinis E, Milios E, Aslanidou M, Mavrokodopoulou O, Smiris P (2009). The effect of stratification on seed germination of *Jasminu fruticans* L.(*Oleaceae*): A contribution to a better insight on the species germination ecology. Int. J. Bot. 5:181-185.
- Rawat DCS, Thapliyal P, Nautiyal AR (1998). Pericarp delays germination in *Quercus glauca* Thunb seeds. J. Trop. For. Sci. 10:472-477.
- Shrestha BB (2003). *Quercus semecarpifolia* Sm. in the Himalayan Region: ecology, exploitation and threats. Him. J. Sci. 1:126-128.
- Singh G, Rawat GS (2012). Depletion of Oak (*Quercus* spp.) Forests in the Western Himalaya: Grazing, Fuelwood and Fodder Collection, Global Perspectives on Sustainable Forest Management, Dr. Clement A. Okia (Ed.), ISBN: 978-953-51-0569-5,
- Suthar AC, Naik VR, Mulani RM (2009). Seed and seed germination in *Solanum nigrum* Linn. Am-Eurasian J. Agric. Environ. Sci. 5:179-183.
- Taiz L, Zeiger E (2002). Plant Physiology, Third Ed. Sinauer Associates, 10:33-46.
- Taylor A, Cosgrove DJ (1989). Gibberellic acid stimulation of cucumber hypocotyl elongation. Plant Physiol, 90:1335-1340.
- Tillberg E (1983). Levels of endogenous abscisic acid in achenes of *Rosa rugosa* during dormancy release and germination. Physiol. Plant. 58:243-248.
- Vazquez C, Rojas M (1996). *Ex situ* conservation of tropical rain forest seed: problems and perspectives. Interciencia, 21(5):293-298.
- Vetaas OR (2000). The effect of environmental factors on the regeneration of *Quercus semecarpifolia* Sm. in central Himalayan, Nepal. Plant Ecol. 146:137-144.
- Wang BSP, Berjak P (2000). Beneficial Effects of Moist Chilling on the Seeds of Black Spruce (*Picea mariana* [Mill.] B.S.P.). Ann. Bot., 86(1):29-36. doi: 10.1006/anbo.2000.1150
- Wilkinson LL (1986). SYSTAT: The system for statistics, Systat, Evanston.
- Zaman S, Padmesh S, Tawfiq H (2011). Selected seed pretreatments on germination of Kuwait's native perennial plant species. Int. J. Bot. 7:108-112.