Effect of pre-germination treatments on seed physiology and germination of central Himalayan oaks?

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ABSTRACT

The continuous decline in regeneration of two important species of central Himalayan oak, namely *Quercus glauca* and *Q. leucotrichophora*, is of great concern. A study was therefore, carried out to improve germination ability of these species using various presoaking treatments. Seeds of both the species lost viability following storage; tetrazolium staining pattern and germination capacity of seeds following different period of storage at 4 °C and 20 °C indicated retainment of viability for a period of 12 months at 4 °C. Of the various physical, chemical and plant growth regulator treatments examined to improve seed germination, only KNO₃ 1.0 % was found to be effective. Seeds scarified at the chalazal end exhibited significant improvement in germination in both the species (94.4 % compared to 56.7 % in control in *Q. glauca* and 82.7 % compared to 64.0 % in control in *Q. leucotrichophora*). The results of this study impart simple methods to improve seed germination for developing nurseries for commercial purposes. [Physiol. Mol. Biol. Plants 2009; 15(4) : 319-326] E-mail : vijaykantpurohit@rediffmail.com

Key words : Central Himalayan oaks, *Quercus glauca*, *Q. leucotrichophora*, Seeds, Germination, Presoaking treatments

INTRODUCTION

Quercus glauca (phaniyat oak) and *Q. leucotrichophora* (banj oak), distributed between 600 and 2000 m amsl (Champion and Seth, 1968), are two important species amongst the 5 evergreen oak species found in the central Himalaya. The common associates of these species are *Pinus roxburghii*, *Myrica esculenta*, *Lyonia evalifolia*, *Rhododendron arboreum*, *Pyrus pashia*, etc. These two oak species occupy an important place in the region because of their significant contribution in soil and water conservation and to sustain rural ecosystems. The wood is used as fuel and for making agricultural tools, leaves as green fodder particularly during lean period and leaf litter as cattle bedding. In order to meet the needs, the species are being harvested indiscriminately from the natural populations. As a

consequence, the forests have either been converted into poor grade oak forest or have been replaced by pine due to an alteration in natural habitat and poor natural regeneration through seeds (Singh *et al.*, 1984; Troup, 1921). Irregular fructification, consumption of seeds by animals (Troup, 1921) and loss of viability during storage for extended periods (Chalupa, 1995) have further overrated the problem of regeneration (artificial and natural). Propagation (clonal propagation) through stem cuttings is difficult in most of the oak species and has not been much successful in these species, moreover, seed weight also influence germination percentage (Purohit *et al.*, 2003). Only marginal success has been reported for cuttings collected from mature trees in *Q. leucotrichophora*, *Q. glauca* and *Q. floribunda* (Tamta *et al.*, 2000, Purohit *et al.*, 2005). In view of these facts, an effort has been made to influence seed germination using different physical, chemical and plant growth regulator (PGR) treatments so as to bring about an improvement in regeneration of these species.

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MATERIAL AND METHODS

Seed Collection

Mature and dry seeds (acorns) of *Quercus glauca* Thunb. and *Q. leucotrichophora* L.A. Camus were collected (hand picked) from Ranman and Katarmal (Dist Almora) area of the central Himalayas, India during early September and in the end of December, respectively (Table 1). Major attributes of seeds of both the species were recorded (Table 1). Seeds were pretreated with Bavistin (0.05 %; w/v) solution for 30 min and wiped dry using blotting paper. Dried seeds were separated from the cupule and kept in open plastic containers at room temperature (22 ± 2 °C; 24 hours) until used for germination trials. All the experiments were carried out in the laboratory and in the Institute nursery at Kosi ($29^{\circ} 37' 30''$ N and $79^{\circ} 37' 15''$ E; 1150 m amsl). Seeds were sown 2.0 cm deep in polybags (16.0 cm h x 8.0 cm dia; one seed per bag) containing a mixture of soil, farmyard manure and sand in the ratio of 3:1:1 (v/v/v). The polybags, kept in open, were watered every alternate day till the experiments completed. Data were recorded daily from the start of germination. All experiments were conducted in a randomized block design (RBD) and each treatment for a given species consisted of 3 sets (each set consisted of 30 seeds; 3 sets x 30 seeds = 90 seeds). The following tests were conducted for detailed investigation:

Viability test

The seed coats were removed carefully from seeds after 24 h soaking in distilled water and submerged completely in 1,2,3 triphenyl tetrazolium chloride (TTC) solution (1 %, w/v; pH 7, 24 h, 25 °C, dark) and per cent viability was determined by counting the total number of stained (red) portions of embryo and cotyledons (I.S.T.A., 1993). Seed viability of both the species was determined for freshly collected as well as for seeds upto 12 months of storage (at 4 and 20 °C).

Imbibition curve

To determine the water imbibition capacity of seeds, initial fresh weight of seeds (45 no.) of each species was recorded. Thereafter, seeds of each set were placed in a beaker containing distilled water (25 °C, dark). Seeds were removed at one hour interval, wiped with blotting paper to remove water from the seed surface and their weight recorded. Following recording of weight, the seeds were transferred back into beakers containing water and the process was repeated till a

constant weight was obtained. An imbibition curve was plotted for an increase in seed weight percentage over initial weight against time.

Treatments to influence germination

(a) Physical

These treatments include: (i) a cut on seed coat at the chalazal end using a sharp scalpel, (ii) Sun drying for 6 hours, (iii) Immersing in hot water at 50 °C for 5, 10 and 15 min, and (iv) Treatment in concentrated H_2SO_4 for 2, 4 and 8 min (Tischler *et al.* 1994). A set of seeds (90 nos.) consisting of 3 replicates was not subjected to these treatments and served as control.

(b) Chemicals and plant growth regulators

To examine the effect of different concentration of chemicals (Thiourea and KNO_3 ; 0.1, 0.5 and 1.0 % from Sisco Research Laboratories (SRL) Pvt. Ltd., India and Qualigens Fine Chemicals) and plant growth regulators (GA_3 and BA; 10, 25 and 250 μM both from Sigma) on germination was used as presoaking treatments. Stock solutions of all chemicals were prepared in water or in 50 % ethanol (v/v) and stored at 4 °C until used; these were diluted with water just before treatment. The final concentration of ethanol in test solutions never exceeded 2.0 % (v/v). The replicate of seeds for each treatment was placed separately in a beaker (250 ml) for 48 h at 25 °C in dark containing appropriate solutions by giving occasional shaking. Treated seeds were subsequently subjected to germination test along with a set of control (untreated) seeds.

Analysis of variance (ANOVA) was performed using the Microsoft excel programme and the level of significance determined following Wilkinson (1986). Least significant difference ($P < 0.05$) was determined for comparison of treatment means as described by Snedecor and Cochran (1967).

RESULTS

The seeds of both the species, i.e., *Quercus glauca* and *Q. leucotrichophora* selected for the present study possessed hard seed coat (Table 1). Based on the mean seed fresh weight, the *Q. leucotrichophora* seeds were heavier than *Q. glauca* along with higher moisture content (*Q. leucotrichophora* = 43.54 %; and *Q. glauca* = 36.44 %). Moreover, seed length and width were also higher for *Q. leucotrichophora*. In both the species the

Table 1. Major attributes of the seeds of *Q. glauca* and *Q. leucotrichophora*.

Attributes	<i>Q. glauca</i>	<i>Q. leucotrichophora</i>
Seed collection site	Ranman (Almora)	Katarmal (Almora)
Altitude (m amsl)	1640	1250
Geographical co-ordinates	29° 32' N and 79° 37' 19" E	29° 36' 10" N and 79° 37' 30" E
Seed maturation time	Aug. – Nov.	Nov. – Jan.
Seed Coat	Hard	Hard
Mean seed fresh weight (g)	17.67±0.32	31.16±5.27
Moisture content (%; FW basis)	36.44	43.54
Mean seed length (mm)	20.41±0.01	22.72±0.97
Mean seed width (mm)	10.97±0.10	13.66±0.86

±: SE, n = 45

seeds started to germinate in and around 60 d and the process was completed within 90 d (results not shown).

Viability test and effect of storage temperature

Freshly collected seeds of both the species were readily stained with TTC (2,3,5-triphenyltetrazolium chloride)

but gradually lost their viability during storage (Table 2). Total staining in *Q. glauca* was 67.7 % for cotyledon and 98.9 % for embryo, while in *Q. leucotrichophora* it was 92.2 % and 97.2 %, respectively. Seeds of both the species lost their viability during storage at both the temperatures i.e., 4 °C and 20 °C, however, the loss was more pronounced at 20 °C and in *Q. glauca* (Table

Table 2. Effect of storage temperature on TTC staining pattern of seeds of *Q. glauca* and *Q. leucotrichophora*.

Species	Months of Storage	TTC staining pattern of (%)			
		4 °C		20 °C	
		Cotyledon	Embryo	Cotyledon	Embryo
<i>Q. glauca</i>	0	67.7±0.00	98.9±0.00	67.7±0.00	98.9±0.00
	3	67.7±1.20	83.5±3.50	62.5±1.07	33.7±1.49
	6	49.1±0.70	41.4±0.70	Not stained	17.8±2.28
	9	45.0±0.80	39.0±2.22	Not stained	Not stained
	12	45.6±1.80	37.0±1.65	Not stained	Not stained
<i>Q. Q. leucotrichophora</i>	0	92.2±0.00	97.2±0.00	92.2±0.00	97.2±0.00
	3	82.7±5.0	92.0±3.72	64.9±8.29	72.3±1.31
	6	66.1±6.02	76.2±3.09	55.8±7.11	68.3±2.02
	9	64.5±1.65	70.8±5.82	44.3±6.69	52.0±2.19
	12	51.2±5.20	62.0±4.34	24.3±4.68	30.9±3.90

±: SE, Each time 45 seeds were used for determining the staining pattern, 15 seeds for one group (n=45)

2). Following storage at 4 °C, staining percentage of cotyledons of *Q. glauca* gradually decreased from 67.7 % (fresh seeds) to 45.6 % (12 months storage); a similar decreasing trend was observed in embryos from 98.9 % (fresh seeds) to 37.0 % (12 months storage). The staining percentage of cotyledons of *Q. leucotrichophora* seeds decreased from 92.2 % (fresh) to 51.2 % (12 months storage) and that of embryos from 97.2 (fresh) to 62.0 % (12 months storage) at 4 °C; a similar trend was observed for both cotyledons and embryo at 20 °C (Table 2). Although TTC staining of embryos of both the species showed 90.0 % staining in freshly harvested seeds, the direct sowing of freshly collected seeds exhibited germinating capacity of 56.7 % for *Q. glauca* and 64.0 % for *Q. leucotrichophora*; the germination capacity also decreased with an increase in the period of storage for both the species at 4 °C and 20 °C (Fig. 1A & B).

Imbibition capacity

The seeds of *Q. glauca* and *Q. leucotrichophora* did not show much variation in their water uptake capacity at 25°C. The seeds imbibed water at a faster rate in the

beginning and attained a saturation level within 48 h of imbibition (Fig. 2).

Physical treatments

Due to the hard seed coat various physical treatments were tried to enhance the per cent germination. Scarification (at the chalazal end) of seeds increased the overall germination percentage. It increased from 56.7 % (control) to 94.4 % in *Q. glauca* and from 64.0 % (control) to 82.7 % in *Q. leucotrichophora*; these values were approximately 40 and 19 % higher in comparison to control (Table 3). The scarified seeds of *Q. glauca* showed early germination success also as highest germination was recorded within 8 weeks and it was completed till 17 weeks, however, in *Q. leucotrichophora* scarification did not show any significant increase in per cent germination upto the first 6 weeks, but after 12 weeks there was a marked enhancement. Germination percentage of seeds subjected to acid scarification with concentrated H_2SO_4 for 2, 4, 8 min was found to be lower as compared to control and it decreased with increasing time of treatment. Drying the seeds in sun for 6 h also lowered the per cent germination when compared to control. Similar to acid scarification and sun drying, germination percentage following dipping the seeds in hot water for 5, 10, and 15 min was found to be lower than control except in *Q. glauca* when 5 min treatment marginally improved the per cent germination; it was lowest for seeds dipped in hot water for 15 min (Table 3). Thus amongst the various physical treatments tried, scarification (cut at chalazal end) was found to be the most effective in this investigation (Table 3). ANOVA

Fig. 1. Effect of storage temperature (4 °C and 20 °C) on seed germination of (A) *Quercus glauca* and (B) *Q. leucotrichophora*.

Fig. 2. Percentage increase in fresh weight over initial weight of *Q. glauca* and *Q. leucotrichophora* seeds at various times after seeds were allowed to imbibe water at 25 °C in the dark.

Table 3. Effect of physical (mechanical) treatments on germination of *Q. glauca* and *Q. leucotrichophora* seeds (final germination response).

Physical treatment	Time	% Germination	
		<i>Q. glauca</i>	<i>Q. leucotrichophora</i>
Control	–	56.7±4.35	64.0±1.33
Sun drying	6 h	51.3±2.51	58.7±0.88
Hot water (50 °C)	5 min	67.8±4.50	60.0±1.33
Hot water (50 °C)	10 min	42.2±2.51	58.7±1.77
Hot water (50 °C)	15 min	32.2±1.52	45.3±1.77
H ₂ SO ₄	2 min	46.7±1.00	62.7±0.88
H ₂ SO ₄	4 min	42.2±1.15	50.7±1.11
H ₂ SO ₄	8 min	33.3±1.00	46.7±1.11
Scarification (cut at chalazal end)	–	94.4±0.57	82.7±1.77
LSD (P < 0.05)	–	22.91	12.67

ANOVA SUMMARY TABLE

Source	DF	MS	F-ratio
Treatments	8	108.185	21.445**
Species	1	8.963	1.777ns
Replication	2	4.241	0.841ns
Treatments × Species	8	18.046	3.577**
Error	34	5.045	

**Level of significance at 0.05; ns-not significant.

showed significant differences in per cent germination of seeds as a consequence of positive and negative effect of different physical treatments. Interaction between physical treatment and species was also found significant at $P < 0.05$ (Table 3).

Chemical treatments

Presoaking treatment of seeds with KNO₃ or thiourea brought only a slight improvement in germination in some cases whereas it was ineffective or inhibitory in others (Table 4). Presoaking treatment of *Q. glauca* seeds with lower concentration (0.1 and 0.5 %) of thiourea resulted in marginal increase, however the

Table 4. Effect of some nitrogenous compounds on germination of *Q. glauca* and *Q. leucotrichophora* seeds (final germination response).

Chemicals	Concentration (%)	% Germination	
		<i>Q. glauca</i>	<i>Q. leucotrichophora</i>
Control	–	56.7±4.35	64.0±1.33
Thiourea	0.10	72.2±3.50	58.7±1.11
Thiourea	0.50	73.3±1.00	68.0±2.66
Thiourea	1.00	35.5±4.93	64.0±0.66
KNO ₃	0.10	50.0±3.26	65.3±2.44
KNO ₃	0.50	40.0±5.35	60.0±0.66
KNO ₃	1.00	81.1±1.52	73.3±1.11
LSD (P < 0.05)	–	26.33	15.17

ANOVA SUMMARY TABLE

Source	DF	MS	F-ratio
Chemical type	1	0.333	0.021ns
Chemical concentration	3	6.806	0.437ns
Species	1	18.750	1.204ns
Replication	2	7.521	0.483ns
Chemical type × Chemical conc.	3	100.722	6.469**
Chemical type × Species	1	5.333	0.343ns
Chemical conc. × Species	3	2.028	0.130ns
Error	33	15.569	

**Level of significance at 0.05; ns-not significant.

higher concentration (1.0 %) was inhibitory (35.5 % compared to 56.7 % in control). In *Q. leucotrichophora*, all the three concentrations (0.1, 0.5 and 1.0 %) of thiourea showed more or less a similar germination range (58.7–64.0 %) as control (Table 4). Seeds treated with lower concentration of KNO₃ (0.1, 0.5 %) showed a decreasing (*Q. glauca*) or similar (*Q. leucotrichophora*) trend when compared with the respective control values. However, with the higher concentration of KNO₃ (1.0 %) an increase in per cent germination (*Q. glauca* 81.1 % and *Q. leucotrichophora* 73.3%) was observed in

Table 5. Effect of GA₃ and BA on germination of *Q. glauca* and *Q. leucotrichophora* seeds (final germination response).

PGRs	Concentration (mM)	% Germination	
		<i>Quercus glauca</i>	<i>Q. leucotrichophora</i>
Control	–	56.7±4.35	64.0±1.33
GA ₃	10	51.1±4.93	69.3±1.77
GA ₃	25	46.7±9.53	74.7±2.44
GA ₃	250	73.3±2.00	77.3±1.55
BA	10	78.9±3.51	66.7±1.77
BA	25	80.0±1.73	61.3±1.11
BA	250	60.0±2.00	70.7±1.55
LSD (P = 0.05)	–	27.94	15.62

ANOVA SUMMARY TABLE

Source	DF	MS	F-ratio
Hormonal type	1	40.333	2.655ns
Hormonal concentration	3	37.250	2.452ns
Species	1	75.000	4.938**
Replication	2	1.750	0.115ns
Hormonal type × hormonal conc.	3	12.389	0.816ns
Hormonal type × Species	1	126.750	8.345**
Hormonal conc. × Species	3	6.500	0.428ns
Error	33	15.189	

**Level of significance at 0.05; ns-not significant.

both the species, although these values were not significantly higher than control (Table 4).

Plant growth regulator (PGR) treatments

Germination percentage of seeds following treatment with different concentrations of two PGRs (GA₃ and BA; 10, 25, 250 mM) is presented in Table 5. In *Q. glauca*, treatments with lower concentrations of GA₃ (10 & 25mM) were not effective and values similar to

control was recorded, but the higher concentration (250 mM) was effective. In *Q. leucotrichophora*, however, all the three concentrations of GA₃ were effective (Table 5). Presoaking treatment with BA, in general, resulted in only marginal improvement in germination in both the species. The improvement in per cent germination with the PGRs examined for both the species were not found to be significantly different (Table 5).

DISCUSSION

Natural regeneration of plant species depends upon the production of viable seeds, subsequent germination and successful establishment of seedlings. Since freshly collected seeds possess viability and are used for raising plants, it is necessary to examine if low temperature storage can be an effective methods of storage for delaying germination. Improper storage conditions do affect viability thus resulting in lower germination percentage. In the present study it was observed that seeds lost their viability during storage as a result of which per cent germination was found to decrease. Assessment of embryo/cotyledon staining pattern and direct germination test indicated that seeds of both the species could be stored for about a year at 4 °C. Hard seed coat do inhibit water uptake resulting in poor germination in this study, nevertheless scarification (cut through chalazal end) could enhance germination in both the species possibly due to immediate absorption of water and enabling the quick emergence of the radicle. The possible role of seed coat as a mechanical barrier (Bewley and Black, 1982) for radicle emergence was excluded because a high germination percentage of seeds occurred when seeds were scarified opposite the micropylar end.

Although the beneficial effect of acid scarification has been reported in some studies (Airi *et al.*, 1998; Das and Saha, 1999), however, in the present study treatment with concentrated H₂SO₄ for varying time periods was not beneficial and found to be detrimental for germination. This is due to corrosive and damaging effects of acid, which possibly penetrates the seed coat, and thus cause damage to the embryo. Such a detrimental effect of concentrated H₂SO₄ has been reported for *Bauhinia* spp. (Upreti and Dhar, 1997). In this study hot water treatment (50 °C) was harmful for seed germination as high temperature results in rapid loss of seed moisture and reduces seed viability (Kandya and Kandya, 1988). Contrary to this, hot water treatments significantly improved the germinability of *Bauhinia racemosa* seeds (Prasad and Nautiyal, 1996). In the

present study sun drying treatment resulted in decreased germination and increased seed mortality probably due to leakage of solutes through the damaged cellular membranes as a consequence of rise in seed temperature.

Besides physical treatments various presoaking treatments were also examined in the present study to optimize seed germination in *Q. glauca* and *Q. leucotrichophora*. The present study suggests that among the various presoaking treatments water soaking of seeds for 48 h was most effective for achieving highest germination. In this study among various plant growth regulators examined, only GA₃ (250 µM) resulted in improved germination in both the species, i.e. *Q. glauca* and *Q. leucotrichophora*. Similarly presoaking treatment with GA also improved germination in *Q. rubra*, and *Q. falcata* (Vogth, 1970; Bonner, 1976) and in other forest tree species, i.e. *Myrica esculenta* (Bhatt *et al.*, 2000). Effect of nitrogenous compounds thiourea and KNO₃ was also examined in this investigation to improve germination. Although thiourea treatment was ineffective in present study, however, Airi *et al.* (1998) reported improvement in some multipurpose tree species of Himalayan region. On the other hand KNO₃ (1.0 %) significantly improved germination in both the species *Q. glauca* and *Q. leucotrichophora*. Similar improvement in germination was reported in *Azadirachta indica* after seeds treated with 1.0 % KNO₃ (Kumaran *et al.*, 1996). The KNO₃ may combine an osmotic role on water uptake with a nutritional effect on protein synthesis. It is used as growth regenerating and germination – stimulating substance in many species (Öztürk *et al.*, 1994).

Based on the staining pattern and germination capacity, it can be concluded that seeds of both the species can be stored for longer period. Among the various treatments tried out (physical, chemical and PGR), only some of the treatments were beneficial to enhance germination percentage in both the species. The most effective treatment in improving germination was found cut at the chalazal end of seeds. This treatment is easy and can be helpful to foresters, nursery growers and local peoples for raising large scale nurseries.

ACKNOWLEDGEMENTS

The financial support from the Department of Biotechnology, Govt. of India is gratefully acknowledged. The Union Ministry of Environment and Forests is thanked for facilities and support.

REFERENCES

- Airi S, Rawal RS, Samant SS and Dhar U (1998). Treatments to improve germination of four multipurpose trees of central sub - Himalaya. *Seed Sci.Tech.* 26: 347-354.
- Bewley JD and Black M (1982) *The physiology and biochemistry of seeds in relation to germination.* Springer-Verlag, Berlin. 2: 170-171.
- Bhatt ID, Rawal RS and Dhar U (2000). Improvement in seed germination of *Myrica esculenta* Buch. - Ham. Ex D. Don – a high value tree species of Kumaun Himalaya, India. *Seed Sci.Tech.* 28: 597–605.
- Bonner FT (1976). Maturation of Shumard and white oak acorns. *Forest Sci.* 22: 149–154.
- Chalupa V (1995). *Somatic embryogenesis in oak (Quercus spp)* In: *Somatic embryogenesis in woody plants.* Jain SM, Gupta PK and Newton RJ (eds), Vol 2, Kulwer Academic Publishers, Dordrecht, The Netherlands, 67-87.
- Champion HG and Seth SK (1968). *A revised survey of the forest types of India.* Govt of India Publ., New Delhi, India, 404p.
- Das B and Saha PK (1999). Effect of dormancy breaking treatments on testa ultrastructures and water uptake patterns of *Albizia procera* seeds. *Seed Sci.Tech.* 27:615-625.
- ISTA (1993) International rules for seed testing. *Seed Sci. Tech.* 21 (supplement).
- Kandya AK and Kandya S (1988). A review of seed research on *Shorea robusta* in India. *Inter. J. Ecol. Enviro. Sci.* 14: 345-356.
- Kumaran K, Surendran C and Palani M (1996). Effect of presowing chemical treatment on germination and seedling growth in Neem (*Azadirachta indica* A. Juss.). *Indian J. For.* 19(1): 87-88.
- Öztürk M, Gemici M, Özdemir F and keyikci M (1994). *Tohum cimlinmesi olayında bitkisel hormonların ve cimlenme stimÜlatörünün tuz stresini azaltmadki rolÜ.* In: XII Ulusal Biyoloji Kongresi Cilt I. Enirne: Ttrakya Universitiesi. pp. 44-48.
- Prasad P and Nautiyal AR (1996). Physiology of germination in *Bauhinia*: involvement of seed coat in inhibition of germination in *B. racemosa* Lam. seeds. *Seed Sci.Tech.* 24:305-308.
- Purohit VK, Palni LMS and Nandi SK (2005). Root formation in stem cuttings of *Quercus glauca* Thunb. and *Q. floribunda* Lindl. : Oaks from the Indian Central Himalaya. *Nati. Aca. Sci. Lett.* 28: 59-61.
- Purohit VK, Tamta S, Nandi SK, Rikhari HC and Palni LMS (2003). Does acorn weight influence seed germination and subsequent seedling growth of central Himalayan oaks? *J. Trop. For. Res. Sci.* 15 (3): 483-492.
- Singh JS, Rawat YS and Chaturvedi OP (1984). Replacement of oak forest with pine in the Himalaya affects the nitrogen cycle *Nature.* 311: 54-56.
- Snedecor GW and Cochran WG (1967). *Statistical Methods.* Oxford and IBH Publishing Co., New Delhi, pp 160–193.

- Tamta S, Purohit VK, Nandi SK and Palni LMS (2000). Chemical induction of root formation in *Quercus leucotrichophora* L. stem cuttings. *Ind. J. For.* 23(2): 135-138.
- Tischler CR, Young BA and Sanderson MA (1994). Techniques for reducing seed dormancy in switchgrass. *Seed Sci.Tech.* 22: 19-26.
- Troup RS (1921). *The Silviculture of Indian Trees*. Vol. III, Clarendon Press, Oxford, p. 1195.
- Upreti J and Dhar U (1997). Study on seed germination of a leguminous liana - *Bauhinia vahlii* Weight & Arnott. *Seed Sci.Tech.* 25: 187-194.
- Vogth AR (1970). Effect of gibberellic acid on germination and initial seedling growth of northern red oak. *For. Sci.* 16: 453-459.
- Wilkinson L (1986). *SYSTAT: The system for statistics*. Eranston, IL: Systat.