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Effect of Stratification Media and Duration on Germination and Seedling Growth of *Quercus glauca* Thunb.

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ABSTRACT

Key words:

Farmyard manure, nutrient content, open nursery condition, shade growing condition Quercu glauca, in lower Himalaya, has restricted occurrence, poor germination and regeneration capacities. Keeping these points in view seven stratification media and three durations were tested to improve germination and performance of seedlings grown under open nursery and shaded conditions. Mean while nutrient content of the soil media was analyzed. Stratification media had significant effect on germination of open nursery conditions but not under shaded conditions. Stratification duration had significant effect on germination, number of lateral roots, shoot height, number of leaves and leaf area of both growing conditions. Later one also had significant effect on collar diameter and root length of seedlings grown under shaded condition only. In general, seedlings grown under shaded conditions had better performance over seedlings grown under open nursery conditions. It was observed that, number of lateral roots, shoot height, and leaf area were higher in 15 days stratification duration; while number of leaves, were higher in 30 days stratification duration and germination per cent was higher in 45 days stratification duration. Thus, it was recommended to use shade growing condition with 15 or 45 days stratification duration to improve seedling growth or germination of the species.

INTRODUCTION

Quercus glauca has wider geographical distribution but restricted occurrence in the Himalayas (Ito et al. 2007, Troup 1921). Oak species, in general, have poor natural regeneration (McCreary 2009, Shrestha 2003, Higo et al. 1995). In addition to biotic factors (Singh and Rawat

2012, Pinero et al. 2010), seed dormancy might have affected germination of these species (Zulfiqar et al. 2015). Stratification, which is the process of treating seeds with cold/chilly condition for certain period of time, is one of the methods used to break seed dormancy. It is mimicry of nature there by seeds germinate well after a season of cold/chilly condition in temperate areas.

Stratification temperature and its duration is an important factor in which many studies had focused on. Most of temperate tree species respond well to cold stratification temperature treatments. But, reports on stratification duration are highly variable. Castro-Colina et al. (2012) reported that acorns of *Quercus rugosa* exposed to 5° C for one week had the maximum germination. Ghildiyal et al. (2009) had also reported that cold stratification (3°C) for 15 days improved the rate and per cent germination of Pinus roxburghii seeds. On the other hand, Fetouh and Hassan (2014) reported that 90 days of cold stratification (5°C) treatment was the most effective stratification duration to improve germination and seedling growth of Magnolia grandiflora. Ghasemi and Khosh-Khui (2007) also reported that 1 to 2 months cold stratification (5°C) increased seed emergence of *Quercus ilex* up to 67%.

Few studies had also focused on the effect of stratification media on germination and seedling growth of tree seeds. Pasquini et al. (2011) recommended the use of polyethylene bag as a stratification container in order to maintain viability of *Quercus ilex* seeds for up to 1 year. Cicek and Tilki (2008) reported that cold stratification for five weeks without media improved germination per cent and germination values of Pterocarya fraxinfolia seeds. In view of the lack of regeneration in its natural habitat and poor germination, the present investigation was aimed at studying the effect of various stratification medias and stratification durations to enhance germination and seedling growth of Quercus glauca.

MATERIALS AND METHODS

The study was conducted at Bogor nursery of Dr. Y.S. Parmar University of Horticulture and Forestry, 173 230 Nauni, Solan (Himachal Pradesh), India. In this study, seven stratification media and three durations has been tested. The tested stratification medias were sand (M_1) , farmyard manure (M_2) , forest soil, collected from *Quercus glauca* forest (M_3) , a 1:1 mix of sand and

farmyard manure (M_4) , a 2:1 mix of sand and farmyard manure (M_{5}) , a 2:2:1 mix of sand, farmyard manure, forest soil (M₆) and local soil, which acted as a control (M_7) . Prior to the stratification trial, nitrogen, phosphorus and potassium analysis of each stratification media was made based on the procedures given by Subbiah and Asija (1956), Olsen et al. (1954) and Merwin and Peach (1951), respectively. In January 2014, viable acorns of Quercus glauca were burried in an open pit filled with the above listed stratification media for 15, 30 and 45 days durations. At the end of stratification periods, acorns were dug out and sown in two homogenous growing conditions (one in open nursery condition and the other in shaded condition). Twenty five seeds from each treatment were sowen on the two growing conditions in RBD factorial design with three replications. Germination was recorded for the first two months. Seeds with visible protrusion of epicotyls on the soil surface were counted as germinated. Other seedling growth parameters were measured at the end of the growing season (8 months young). In that five seedlings from each treatment were randomly taken for the measurement of growth parameters.

RESULTS AND DISCUSSION

Open nursery condition

Data pertaining to various germination traits of *Quercus glauca* seedlings has been depicted under table 1. The germination in farm yard manure (M_2) was high followed by forest soil (M_3) and a 2:2:1 mix of sand, farmyard manure and forest soil (M_{e}) having values of 51.8 %, 49.3 % and 48.9 %, respectively. The least germination per cent (44.0%) was recorded in the control (M_{τ}). This indicates that germination was highest in seeds stratified in media having higher fertility status (Figure 1). In contrary to this, Dolor (2011) and Pahla (2014) recorded higher germination in the seeds stratified under nutrient poor soil media. Nutrient content of the stratification media is not expected to influence germination. Rather the high moisture holding capacity and high amount of micro-organisms available in fertile media might have indirect impact on germination (Araujo et al. 2009, Wall 2005, Hanapi et al. 2014, Jalaluddin and Hamid 2011). Apart from this germination per cent, stratification media had no significant effect on the growth of *Quercus glauca* seedlings.

Stratification duration had a significant effect on both germination per cent and growth traits of seedlings *viz.*, number of lateral roots, shoot height, number of leaves, and leaf area. Seedlings in the fifteen days stratification duration (D_1) has the highest number of lateral roots (0.67) and rootshoot ratio (0.58). Whereas, seedlings in the thirty days of stratification duration (D_2) has the highest values of shoot height (9.3 cm) and number of leaves (2.9). Forty five days of stratification duration (D_3) has the highest germination per cent (49.5 %). In line with this study, Drake and Ewing (1997) reported that six weeks stratification duration had significant effect on germination of several native Washington plant species. The importance of moderate stratification period for germination and seedling growth of trees is reported by various authors (Fetouh and Hassan 2014, Ghasemi and Khosh-Khui 2007). However, Pandey and Tamta (2013) reported 10 to 20 days stratification duration have better germination of *Quercus serrata* and *Quercus semecarpifolia* acorns. The interaction effect of stratification media and duration on germination and seedling growth of *Quercus glauca* under open nursery condition was found insignificant.

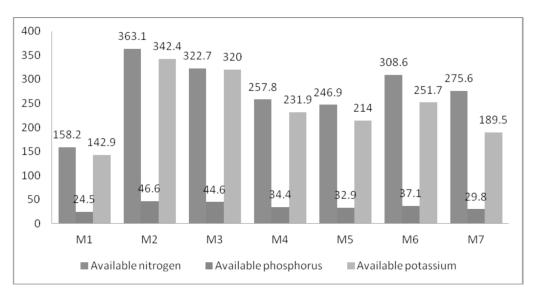


Figure 1. Soil nutrient content (kgha⁻¹) of stratification media

Shaded growing condition

Stratification media failed to exercise significant effect on germination and growth of *Quercus glauca* seedlings grown under shade (Table 2). However, stratification duration had significant effects on germination and growth of seedlings. Seeds stratified for 15 days stratification duration (D_1) produced seedlings with higher collar diameter (0.28 cm), root length (16.1 cm), shoot height (14.4 cm) and leaf area (10.3 cm²). Whereas, seeds stratified for 30 days stratification duration (D_2) developed seedlings which have higher collar diameter (0.28 cm), number of lateral roots (0.85) and number of leaves (2.8). While, the 45 days stratification duration (D_3) had significantly higher (66.9 %) germination per cent,. Differences on survival per cent, root- shoot ratio and total biomass were insignificant. In contrary to this result, Fetouh and Hassan (2014) reported higher root, shoot, leaf, and vigour index of *Magnolia grandiflora* seedlings under 60 to 90 days stratification treatment.

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of strat	grown under open nursery condition
Table 1. Effect	grown un

Treatments	Germination (%)	Survival (%)	Collar diameter (cm)	Root length (cm)	Number of lateral roots	Shoot height (cm)	Root- Soot ratio	Number of leaves	Leaf area (cm²)	Total biomass (g)
Stratification media	n media									
M_1	42.0 (44.9)	51.8 (61.6)	0.27	13.4	0.25	8.7	0.61	2.5	3.6	0.34
M_2	46.0 (51.8)	47.5 (54.3)	0.29	13.8	0.39	8.6	0.47	2.8	4.1	0.40
M_3	44.6 (49.3)	48.3 (55.7)	0.28	13.3	0.30	9.3	0.52	2.6	3.8	0.30
${ m M}_4$	42.3 (45.3)	51.2 (60.5)	0.29	13.8	0.62	8.7	0.60	2.8	3.7	0.35
M_5	42.0 (44.9)	53.1 (63.9)	0.29	14.2	0.31	9.2	0.56	2.3	3.8	0.32
M_6	44.3 (48.9)	55.1 (66.9)	0.29	14.7	0.49	8.4	0.64	2.4	3.7	0.32
M_7	41.5 (44.0)	53.3 (63.9)	0.27	13.3	0.43	8.4	0.59	2.6	3.5	0.31
SE+	1.04	1.85	0.01	0.59	0.14	0.38	0.20	0.18	0.26	0.03
CD 0.05	2.981	NS	SN	SN	SN	SN	NS	SN	SN	NS
Stratification duration	n duration									
D_1	40.5 (42.3)	49.8 (58.1)	0.28	13.6	0.67	8.9	0.58	2.3	4.3	0.35
D_2	44.5 (49.2)	53.4 (64.2)	0.29	13.4	0.34	9.3	0.55	2.9	3.6	0.33
D_3	44.7 (49.5)	51.2 (60.6)	0.28	14.3	0.19	8.1	0.57	2.6	3.2	0.32
SE+	0.68	1.21	0.01	0.39	0.09	0.25	0.13	0.12	0.17	0.02
CD 0.05	1.95	NS	NS	NS	0.26	0.71	NS	0.34	0.49	NS
Figures in parenthesis are ori	nthesis are orig	ginal values								

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The interaction effect of stratification media and duration on germination and seedling growth parameters under shade condition was found insignificant.

Comparative analysis of open and shaded growing conditions

The two growing conditions differed in their performance of germination and seedling growth parameters (Table 3). Results from figure 2, 3 and 4 showed that the average values of germination per cent and seedling survival was 15.3 % and 33 % more under shade growing condition than open nursery conditions, respectively. Average values of root length, number of lateral roots, shoot height and leaf area were also higher in shade growing condition with respective advantages of 1.3 cm, 0.17, 4.7 cm and 4.7 cm² over open growing condition. The average values of collar diameter, root-shoot ratio, number of leaves and total biomass were higher in open nursery condition with respective advantage of 0.1 cm, 0.23, 0.1 and 0.06 g over shade growing condition.

Moisture and light condition were the two important factors that influenced germination and seedling growth under the two growing conditions. The availability of constant and sufficient moisture under shade growing condition enhanced germination and survival by reducing desiccation of young seedlings. Under shade growing condition, roots were thin and long, low in biomass, and developed more lateral roots. Shoots became thin and long and low in biomass while leafs became few in number with sufficiently large leaf area to capture intermittent light that passed through shades. Gottshalk (1985) reported that height of red oak and black oak seedlings were the highest in 80% shading treatment whereas, their diameter decreased along with increase in shading. Root-shoot ratio was also decreased with low light intensity. Muick (1991) also reported better emergence and survival of blue oak and coast live oak under 50 % shade field condition than open condition.

Under open nursery condition, emerging shoots was frequently observed dying and seedlings responded by coppicing or developing multiple stems. This might be the reason for higher collar diameter and shorter shoot heights of these seedlings. Roots were shorter and thicker in open nursery condition that influenced root-shoot ratio and total biomass to be higher. Moisture stress, due to high evapo-transpiration, might be a reason for the growth of shorter and thicker roots. Leaves were also thin and narrow but more in number. Direct sun light might have affected the growth and enlargement of these leaves. But the high number leaves might have helped them to compensate the low leaf surface area of individual leaves.

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Treatments	Germination (%)	Survival (%)	Collar diameter (cm)	Root length (cm)	Number of lateral roots	Shoot height (cm)	Root- Soot ratio	Number of leaves	Leaf area (cm²)	Total biomass (g)
Stratification media	n media									
\mathbf{M}_1		76.9								
	51.1 (59.6)	(200.7)	0.27	14.4	0.50	13.3	0.32	2.3	8.4	0.24
${ m M}_2$		80.8								
	53.6 (64.4)	(63.9)	0.28	15.1	0.47	14.5	0.31	2.7	8.0	0.26
${ m M}_3$		85.0								
	55.5 (67.1)	(97.2)	0.27	15.2	0.48	13.4	0.43	2.3	8.3	0.28
${ m M}_4$		84.4								
	51.0 (60.2)	(96.3)	0.28	14.6	0.57	12.7	0.36	2.5	8.0	0.31
\mathbf{M}_{5}		77.6								
	49.6 (57.8)	(91.7)	0.27	14.8	0.41	13.1	0.32	2.6	8.2	0.27
${ m M}_6$		74.8								
	57.2 (69.3)	(90.4)	0.27	15.8	0.80	14.3	0.34	2.4	9.1	0.28
\mathbf{M}_7		85.7								
	49.4 (57.6)	(98.1)	0.28	15.6	0.74	13.3	0.32	2.4	9.8	0.28
SE+	2.49	3.91	0.01	0.96	0.16	0.42	0.05	0.18	0.70	0.03
CD 0.05	NS	NS	NS	NS	NS	NS	NS	SN	SN	SN
Stratification duration	n duration									
D_1		84.6								
	49.2 (57.2)	(97.6)	0.28	16.1	0.72	14.4	0.29	2.5	10.3	0.28
D_2		78.1								
	52.8 (62.8)	(91.3)	0.28	15.6	0.85	13.0	0.37	2.8	8.5	0.29
D_3		79.6								
	55.5 (66.9)	(63.3)	0.26	13.5	0.13	13.2	0.37	2.2	6.9	0.25
SE+	1.63	2.56	0.00	0.63	0.11	0.28	0.03	0.12	0.46	0.02
CD 0.05	4.668	NS	0.010	1.804	0.302	0.794	SN	0.344	1.322	NS
Figures in parent	Figures in parenthesis are original values	values								

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Table 3. Mean square analyses of germination and growth parameters for the different growing conditions, stratification media, duration and their interactions

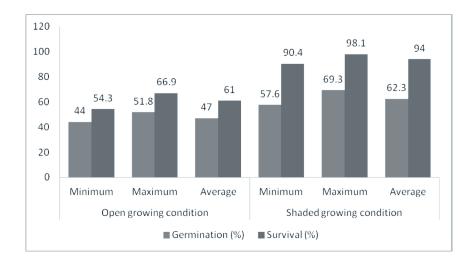
Sources	df	Germination %	Survival %	Collar diameter	Root length	Number of lateral roots	Shoot height	Root- Shoot ratio	Leaf number	Leaf area	Total biomass
Growing condition (G)	1	7344.8^{**}	34463.4^{**}	0.00**	51.3^{**}	0.9^{*}	709.0^{**}	1.6^{**}	0.32	731.2^{**}	0.11**
Stratification media (M)	6	240.1^{*}	87.0	0.00	3.3	0.3	1.6	0.02	0.35	1.3	0.01
Stratification duration (D)	2	803.3**	9.7	0.00	9.5	3.4^{**}	11.1**	0.01	2.56^{**}	54.1**	0.01
$G \times M$	6	36.7	194.8	0.00	1.6	0.08	3.3	0.03	0.25	2.9	0.01
$G \times D$	2	38.9	400.7^*	0.00	33.8^{**}	0.95^{*}	9.0^{*}	0.04	1.14^{*}	14.3^{**}	0.00
$M \times D$	12	146.8	108.7	0.00	2.6	0.26	2.1	0.03^{*}	0.63^{*}	2.8	0.01
$G\times M\times D$	12	143.2	59.5	0.00	2.7	0.38	2.0	0.01	0.30	3.8	0.01
Error	84	81.2	94.3	0.00	5.8	0.20	2.1	0.02	0.32	2.7	0.01
Mean		54.7	77.5	0.28	14.4	0.5	11.1	0.5	2.5	6.1	0.3
(SE <u>+</u>)		1.2	1.7	0.0	0.2	0.0	0.3	0.0	0.1	0.3	0.0

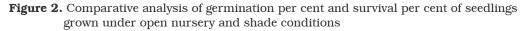
* Significant at the 0.05 level (2-tailed)

** Significant at the 0.01 level (2-tailed)

Table 4. Mean comparison of germination and growth parameters by Tukey's Honestly significant difference test

					Growth para	meters				
Stratification duration	Germinat ion %	Surviv al %	Collar diameter	Root length	Number of lateral roots	Shoot height	Root- Shoot ratio	Leaf number	Leaf area	Total biomass
15	49.7^{b}	77.9	0.29	14.9	0.71 ^a	11.7^{a}	0.43	$2.4^{ m b}$	$7.3^{\rm a}$	0.31
30	56.0^{a}	77.7	0.29	14.5	0.62^{a}	11.2^{ab}	0.46	2.8^{a}	6.1^{b}	0.31
45	$58.2^{\rm a}$	77.0	0.28	13.9	$0.17^{ m b}$	$10.6^{\rm b}$	0.47	$2.4^{ m b}$	5.1°	0.28
$HSD_{0.05}$	0.00	0.91	0.34	0.17	0.00	0.01	0.54	0.00	0.00	0.35
SE	1.97	2.1	0.01	0.53	0.10	0.31	0.03	0.13	0.36	0.02





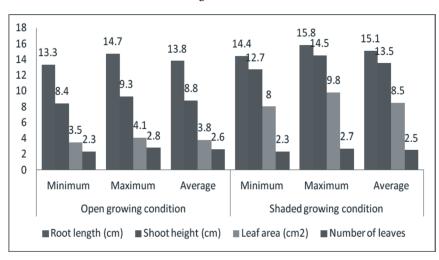


Figure 3. Comparative analysis of root length, shoot height, leaf area and number of leaves of seedlings grown under open nursery and shade conditions

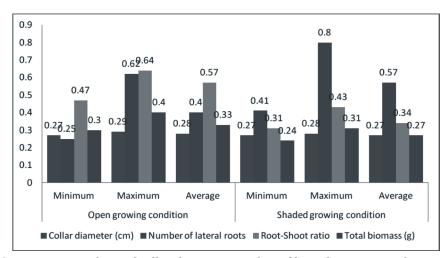


Figure 4. Comparative analysis of collar diameter, number of lateral roots, root-shoot ratio and total biomass of seedlings grown under open nursery and shade conditions

In general, stratification media had no significant effect on the growth of *Quercus glauca* seedlings, except germination of acorns (Table 3). Stratification duration had a significant effect on germination, number of lateral roots, shoot height, leaf number and leaf area. Growing media, on the other hand, had a significant effect in all parameters except leaf number. Mean comparison by Tukey's Honestly Significant Difference (HSD_{0.05}) test failed to differentiate impact of stratification media in all germination and growth parameters. However, seedlings in 15 days stratification duration had significantly higher number of lateral roots, shoot height and leaf area while seedlings in 30 days stratification duration had significantly higher number of lateral roots.

higher number of leaves and acorns in the 45 days stratification duration had significantly higher germination per cent (Table 4).

CONCLUSION

It can be concluded that regardless of stratification media, 45 days stratification duration had improved germination while 15 days stratification duration had better seedling growth performance. The use of shaded growing condition also improved germination and growth of *Quercus glauca* seedlings in the first growing season. However, it is advisable to consider reports from Welander and Ottosson (1998, 2000) that stated initial growth of oak seedlings was not affected by low light condition, but proper growth of oak seedlings would be achieved by increasing light intensity after one year.

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