SEED STORAGE AND GERMINATION STUDIES IN BLUE PINE

K. M. Siddiqui and Mohammad Parvez

Abstract. *This paper describes results of studies on seed storage and germination of Himalayan blue pine. The effects of seed storage and germination temperatures and stratification, insecticidal and fungicidal treatments on germination of blue pine seed were determined for different seed sources of this species in these studies. It was observed that the viability of blue pine seed is significantly reduced within short period if it is stored at high temperature. Seed stored in dry cold or stratified condition retains its viability and maximum germination per cent as well as speed of germination are obtained if the seed is stratified for 120 days. Insecticidal treatment of seed in storage adversely affects its germination. Large differences were also noticed between different seed sources of blue pine in respect of various germination characteristics.*

Introduction. Tree species exhibit seeding periodicity and seeding may vary from very poor to very good in different year. In a study of seeding periodicity in Himalayan blue pine *(Pinus wallichiana* A.B. Jacks. Syn. *P. griffithii),* in its natural range in Pakistan and Azad Kashmir, it was found that good seed years are not common (Siddiqui and Pervez, 1979). Under these circumstances, it is essential that enough seed be collected and stored during good seed years to meet its requirement in lean seed years. However, information is lacking on optimum storage conditions for blue pine seed. According to Wang (1974) and Stein, *et. al.*, (1974) , the storability of tree seed is affected by many factors. The most critical factors are; type of seed, stage of maturity, prestorage treatment, viability, moisture content, air-temperature, humidity and oxygen pressure and degree of infection by insects and fungi during storage. Because of differences in physical and physiological characteristics of seed, the effect of each factor on seed storability also varies with tree species.

Research on seed germination has been largely concerned with dormancy. A seed that does not germinate in a properly conducted test or in the nursery may be alive or dead. If it is alive, it is dormant. Considerable research has been conducted with finding practical methods for breaking seed dormancy (Bonner, *et, al.*, 1974). Further, the time seeds need to germinate is also important. Various treatments are generally applied to hasten germination e.g. scarification, stratification, water soaking, high temperature, chemical and excision of embryos. Our experience in the past had shown that cold stratification of blue pine seed is needed for satisfactory germination in the nursery.

This paper describes results of studies on seed storage and germination of blue pine carried out under a PL-480 Project of U.S. Department of Agriculture.

The authors are Director, Forest Products Research Division and Research Assistant respectively at the Pakistan Forest Institute, Peshawar.

The effects of storage temperature, germination temperature, stratification treatment and insecticidal and fungicidal treatments on germination of blue pine seed were determined for different seed sources in these studies.

Material and Methods. Seed from different individual trees and sources of blue pine was cleaned to separate empty seed from the filled seed. Four samples each consisting of 50 seed from each seed lot were germinated in petri dishes containing sand at 25 + 5°C in a germinator except in germination temperature study in which temperature was variable. Petri dishes and sand were sterlized before the start of germination test. Artificial light was provided inside the germinator for six hours every day. The germinates were counted daily as soon as the radicles emerged from the seed coat and then were removed from the petri dish. The tests were run for 40 days and the ungerminated seed at the end of the test period was cut to determine the number of empty, rotten and sound seed. The number of sound seed after completion of germination were added to those germinated to determine germination capacity. The speed of germination was measured as the number of days to reach 60% of the total germination in each test sample. In those tests in which the germination per cent of all seed sources did not reach 60%, the limit was reduced to 40%.

Germination data was transformed to arcsin and square root and then analysed by factorial analysis of variance methods suggested by Steel and Torrie (1960) to determine significant differences of germination among and within seed sources of blue pine as well as between various treatments applied to the seed before germination tests. Dunnett's (1955) procedure was followed for comparison of sample means.

Results and Discussion. The results of different studies conducted on storage and germination of blue pine are discussed below:

a. Effect of long duration of storage on germination of blue pine seed.

Blue pine seed from two sources of Gharial and Patriata in Murree Forest Division and stored upto three years at room temperature (10 to 30 °C) was tested. Percent germination was found to decline sharply with increased duration of storage under these conditions. Highest germination was obtained in seed stored for one year which reduced to almost nil after three years storage of seed as shown in Table 1. The seed of other seed sources which was stored for upto ten years did not germinate at all. Normally, the viability of naturally dispersed blue pine seed extends into second growing season under sub-freezing conditions prevalent in the forests during winter. According to Barton (1961), the viability of tree seed can be maintained at low temperature and humidity for upto 15 years The storage temperature for blue pine seed of the present study was very high and this might be the reason for sharp decline in its viability with time.

Table 1

Germination per cent of blue pine seed from Gharial and Patriata stored at room temperature for variable periods.

Period of storage	Seed sources	
	Gharial	Patriata
One year	69.5	48.6
Two years	9.7	20.5
Three years	1.0	0.3

b. Effect of storage temperature on germination of blue pine seed. The above study of the effect of long duration of storage on germination of blue pine seed was a preliminary study showing reduction in germination with increase in period of storage. Another study was carried out to determine the influence of storage temperature on germination of blue pine seed. In this case, seed of 8 seed sources of Dunga, Kalam, Kumrat, Ushu, Madaglas, Utror, Shringal and Bamburet from Dir, Swat, Chitral and Hazara Tribal Forest Divisions were tested in fresh condition as well as after storage for eight months at 2 + 2°C both in stratified and unstratified conditions and at room temperature of 10-30 °C and 10-42 °C. A split plot design was used for the experiment. The results are summarised in Table 2.

The differences of germination %, germination capacity and speed of germination were found to be highly significant between all seed sources and storage temperature treatments and their interaction. Germination was significantly decreased with high storage temperature. Except for the seed from Bamburet from Chitral, the germination of seed of all other sources was very low after storing them at room temperature. The results of this study indicate that blue pine seed can be stored in dry cold or stratified condition with no loss in germination. Storage at room temperature is not desirable.

c. Effect of provenance and stratification on germination of blue pine seed. It was commonly observed in the past that blue pine seed germinates well only if it is stratified at low temperature before sowing in the nursery. Therefore, experiments were conducted to determine optimum duration of stratification treatment for this seed. Seed from 8 seed sources, mentioned in the above study, was stratified in moist sand at 2+2 °C for 20, 40, 72 and 120 days. An equal quantity of seed of these seed sources was also stored at the same temperature without stratification treatment e.g. The seed was not stored in moist sand. A split-split plot design was followed in this study. The results are presented in Tables 4, 5 and 6 for germination %, germination capacity % and speed of germination respectively.

Table 2

Germination % (G), germination capacity % (Gc) and speed of germination in days (Sg) of blue pine seed stored at different temperatures.

Seed source	Fresh			Stratified 2+2 °C			Dry cold 2+2 °C			Room 10-30 °C			Room 10-42 °C		
	G	Gc	Sg	G	Gc	Sg	G	Gc	Sg	G	Gc	Sg	G	Gc	Sg
Dunga	66	66	20	100	100	4.5	100	100	4.5	28	28	—	24	24	—
Kalam	95	95	4.5	98	98	3	83	87	7	9	9	—	14	14	—
Kumrat	96	96	7.5	94	98	4	59	59	8	27	27	—	24	24	—
Madaglas	95	97	5	97	98	4.5	88	88	8	46	46	—	39	43	—
Utror	91	91	3.5	100	100	3	98	98	6.5	6	6	—	7	7	—
Shringal	72	79	10	91	91	3.5	64	70	22	20	20	—	13	13	—
Bamburet	96	100	5.4	96	100	4	64	64	12.5	89	89	7	85	85	10.5
Ushu	90	90	7.5	98	100	4	98	100	8	25	25	—	22	22	—
Average	86.9	89	7.9	97.2	98	3.8	81.7	83.2	9.6	31	31	—	28.2	30	—

Table 3

F-values of analysis of variance for study of effect of storage temperature on germination of seed from different sources of blue pine.

Source of variation	df	F-values Germination %	Germination capacity %	Speed of germination
Blocks	1	n.s 3.31	n.s. 2.82	** 40.5
Seed sources A	7	** 12.07	** 13.01	** 68.00
Error (a)	7			
Seed treatments B	4	** 378.07	** 405.60	** 73.00
Interaction AB	28	** 13.94	** 12.82	** 11.20
Error (b)	32			

n.s. — non-significant
** — highly significant

Table 4

Effect of cold storage and stratification on germination % of blue pine seed.

Seed source	20 days CS	20 days S	40 days CS	40 days S	72 days CS	72 days S	120 days CS	120 days S
Dunga	84	54	100	82	72	83	94	100
Kalam	78	76	94	92	78	98	82	100
Ushu	97	94	86	99	69	94	93	100
Madaglas	90	100	85	99	68	96	66	100
Bamburet	100	94	96	85	66	87	89	97
Shringal	81	55	97	86	77	68	69	90
Utror	92	72	100	100	79	98	94	99
Kumrat	92	96	67	61	76	96	69	96
Average	89.2	80.1	90.6	88.0	73.0	90.0	82.0	97.7

CS — Cold storage S — Stratification

Table 5

Effect of cold storage and stratification on germination capacity % of blue pine seed.

Seed source	20 days		40 days		72 days		120 days	
	CS	S	CS	S	CS	S	CS	S
Dunga	85	62	100	82	78	86	94	100
Kalam	79	76	94	93	91	98	83	100
Ushu	97	94	89	99	79	94	93	100
Madaglas	90	100	87	99	80	97	66	100
Bamburet	100	94	96	88	80	91	89	97
Shringal	89	64	98	88	84	79	69	90
Utror	96	72	100	100	85	98	94	99
Kumrat	92	96	83	85	86	98	69	100
Average	91	82	93	92	83	93	82	98

CS — Cold storage S — Stratification

Table 6

Effect of cold storage and stratification on speed of germination of blue pine seed.

Seed source	20 days		40 days		72 days		120 days	
	CS	S	CS	S	CS	S	CS	S
Dunga	11	26	10	18	25	12	11	5
Kalam	27	9	13	7	12	7	11	1
Ushu	10	10	11	6	15	7	9	4
Madaglas	10	9	10	7	24	7	11	4
Bamburet	7	9	8	9	28	9	13	5
Shringal	6	22	9	9	17	9	11	4
Utror	8	16	7	4	13	7	11	3
Kumrat	8	9	26	31	19	9	12	11
Average	11	14	12	11	19	8	11	4

CS — Cold Storage S — Stratification.

As shown in Table 7, the F-values of analysis of variance are mostly highly significant for differences of germination %, germination capacity % and speed of germination between different seed sources and their interaction with stratification treatment and duration of treatment. Both treatment and its duration did not have any significant effect on germination percent and germination capacity. However, on the whole stratified seed showed a higher germination % as compared to unstratified seed. Seed stratified for 120 days showed the best germination. The speed of germination was significantly improved after stratification treatment.

Table 7

F-value of analysis of variance of the effects of cold storage and stratification on germination of blue pine seed from different sources.

Sources of variation	df	F-values		
		Germination %	Germination capacity	Speed of germination
Blocks	1	n.s 0.04	n.s. 0.15	n.s. 0.95
Periods	3	n.s. 4.04	n.s. 1.89	* 16.21
Error (a)	3			
Treatments	1	n.s. 2.15	n.s. 2.46	n.s. 2.08
Periods X Treatments	3	n.s. 1.88	n.s. 2.75	n.s. 0.89
Error (b)	4			
Sources	7	** 7.03	** 9.83	** 3.95
Periods X Sources	21	** 5.09	** 6.80	** 2.37
Treatments X Sources	7	** 12.31	** 13.06	** 2.51
Periods X Sources X Treatments	21	** 16.36	** 2.40	n.s. 0.98
Error (c)				

n.s. — non-significant * — significant ** — highly significant

Table 8

Effect of germination temperature on germination % (G) and germination capacity % (Gc) on germination of blue pine seed.

Seed source	Germination temperature °C			
	15	20	25	30
	G	G	G	G
Hillan	80	85	76	68
Thandiani	73	73	42	18
Kalam	59	86	—	—
Ushu	55	91	60	48
Sathangali	55	92	46	29
Average	64.4	85.4	56.0	49.6

d. Effect of germination temperature on germination of blue pine seed. Blue pine seed from 5 seed sources, namely, Hillan, Thandiani, Kalam, Ushu and Sathangali from Swat, Hazara Tribal, Galis and Siran Forest Divisions was tested at germination temperature of 15, 20, 25 and 30°C. Results of germination % of these seed sources at different temperatures are given in Table 8. F-values in analysis of variance were found to be significant at 5% probability level for differences between various temperature treatments. These values were 4.260 for germination %. Fairly high germination was obtained in all seed sources at low temperature of 20°C. This study shows that blue pine seed can be sown in the nursery fairly early in the spring when the day temperature is around 20°C.

Table 9

Germination of treated and untreated blue pine seed.

Treatment	Germination %	Germination Capacity %	Speed of Germination in days
Arasan prior to storage	54	61	40
Arasan after storage	51	56	36
Sevin after storage	28	29	42
Arasan and Sevin after storage	27	33	46
Untreated fresh seed	53	55	29

e. Germination of blue pine seed treated with insecticide and fungicide during storage. Blue pine is generally treated with insecticide 'Sevin' and fungicide 'Arasan' to protect it from insects and fungi attack during storage. However, it was observed in the past that seed treated with 'Sevin' gave poor germination. Therefore, tests were conducted to determine the effect of the treatments of 'Arsan' and 'Sevin' on germination of seed stored in dry cold condition (2—4°C) for eight months. A single seed source of blue pine was tested in this study. The results are given in Tables 9 and 10. Sevin-treated seed showed significantly poor germination as compared to other treatments.

Table 10

F-values of analysis of variance for effects of fungicide and insecticide on germination of blue pine seed.

Character	Source of variation	df	F-values
Germination %	Blocks	3	1.86 n.s.
	Treatments	4	9.36 **
	Error	12	
Germination capacity %	Blocks	3	1.53 n.s.
	Treatments	4	11.1 **
	Error	12	
Speed of germination	Blocks	3	0.84 n.s.
	Treatments	4	2.60 n.s.
	Error	12	

n.s. — non-significant ** — highly significant

Conclusion. The results of above studies have indicated the storability and germination characteristics of blue pine seed. These results are of great practical importance. The seed of blue pine should be stored in dry cold condition to retain its viability. The viability of seed would be significantly reduced within one year if it is stored at room temperature. A suitable insecticide is also required for seed treatment prior to its storage. Stratification treatment has been found to be essential in the experiments for optimum germination; the latter being dependent upon duration of treatment. Maximum germination per cent and speed of

germination is obtained when the seed is stratified in moist sand for 120 days. Blue pine seed germinated satisfactorily at low temperature as well. Further, germination characteristics vary between different seed sources of this species to a significant extent. Therefore, the selection of a seed source of blue pine for afforestation purpose will depend upon its germination characteristics besides general ecological factors of the locality of seed collection. Nursery sowing of blue pine seed can be successfully carried out early in the spring when soil temperatures are 15-20°C and it should be preceded by stratification treatment of the seed for sufficient period.

LITERATURE CITED

1. Barton, L. V. 1961. Seed preservation and longivity. Inter Science Publishers, New York.

2. Bonner, F. T., B. F. Mclemore, and J. P. Barnett. 1974. Presowing treatment of seed to speed germination. *In.* Seeds of Woody Plants in the United States. Forest Service, U.S. Department of Agriculture, Agriculture Handbook No. 450, 127-134.

3. Dunnet, C. W. 1955. A multiple comparison procedure for comparing several treatments with control. Jour. Am. Stat. Assoc. 50: 1096-1121.

4. Siddiqui, K. M. and M. Parvez. 1979. Seeding periodicity in blue pine. Pak. Jour. For. 29(2): 87-89.

5. Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co. New York. 481 pp.

6. Stein, W. I., P. E. Slabaugh and A. P. Plummer. 1974. Harvesting processing, and storage of fruits and seeds. *In.* Seeds of Woody Plants in the United States. Forest Service, U.S. Department of Agriculture, Agriculture Handbook No. 450:98-121.

7. Wang, B. S. P. 1974. Tree seed and pollen storage for genetic conservation; possibility and limitations. FAO/UNDP Report on Pilot Study on the Methodology of Conservation of Forest Genetics Resources. FAO Misc. Publ. 75/8. FAO, Rome, Italy: 93-103.