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Storage of *Pinus gerardiana* Seeds: Biochemical Changes and its Applicability as Vigour Test

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Abstract: Standardization of storage condition is prerequisite for management of Endangered *Pinus gerardiana* species. Therefore, the present study was undertaken to deals with the effect of storage temperature and type of containers on viability, moisture content and biochemical parameters and changes in them during different time periods (3, 6, 9 and 12 months) of seed storage. For standardization of storage device, the seeds were stored at four temperatures viz., room temperature (19-22°C), 0±1, -4±1 and 4±1°C in five different containers viz., polythene bags, plastic jars, canvas bags, earthen pots and tin boxes. The study revealed that there was a slight decrease in viability of seeds from fresh to nine month period when stored at 0±1°C in earthen pots. The S_2C_4 storage condition performed significantly ($p \leq 0.05$) better by achieving maximum viability of 94.30, 92.33, 84.83 , 65% and other biochemical parameters after 3, 6, 9 and 12 months of storage period. The storage devices S_1C_5, S_2C_3, S_3C_3 and S_3C_4 for 3 months, S_2C_3 and S_3C_4 for 6 and 9 months also shown significantly ($p \leq 0.05$) good seed vigour during the period of storage. This is associated with decrease of moisture and oil per cent but increase of acid value and refractive index of oil content. The total sugar and soluble proteins were however found to increase up to three months after which there was a progressive decline as the storage period was extended up to 12 months. The results indicated that combination of 0±1°C x earthen pot is better storage device for *Pinus gerardiana* seeds storage. The implementation of standardized storage condition, conserve the viability of seeds for conservation of species through plantation.

Key words: Biochemical's, endangered, Himalayan, *Pinus gerardiana*, storage, viability

INTRODUCTION

Storage is an important aspect of any sound seed management programme especially in conifer species where it is necessary practice due to short supply and dormancy of seeds (Magini, 1962; Vlase, 1974; Sharma *et al*., 2004). *Pinus gerardiana* Wall. commonly known as chilgoza or neoza pine is an important species of dry temperate zone and occupies a place of pride due to its economic product in the form of edible seeds (Sehgal and Khosla, 1986). The local people have got the right of seed collection from natural forest and selling them in the market at high rates ranging from Rs.400-650 kg^{-1}. In this way, each and every cone is

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lopped, leaving very little number of seeds for natural regeneration (Tandon, 1963; Singh *et al*., 1973). The species is thus facing extinction and has been included in the Red Data Book and categorized as an endangered species (Sehgal and Sharma, 1989). Besides, the species has erratic and infrequent seed years and dormancy related problem which also prevents its regeneration in natural habitat (Malik and Shamet, 2008; Malik *et al*., 2008). The seeds being highly nutritious also get severely infested by various fungi and insects during storage and transportation (Singh and Gupta, 1989). Moreover, it loses viability more rapidly as compared to other conifers. Standardization of container type and temperature condition is thus a prerequisite for long term storage to facilitate nursery raising (in lean period) and seed handling during storage and shipment (Allen, 1962; Robbins, 1984). Keeping these points in view, studies on storage temperature and types of containers along with the biochemical changes were under taken to understand and conserve viability of the seed to meet plantation target in the species.

MATERIALS AND METHODS

The present study was conducted in the Department of Silviculture and Agroforestry, Dr. Yspuhf, Nauni-Solan under laboratory. The seeds were collected from forests of Kalpa and Pangi villages situated at 31°55' to 32°05' N latitude and 77°45' to 79°35' E longitude during October-November of 2004 and 2005 each. The fresh seeds were packed in gunny bags and transported to University campus to test longevity of the seeds. Prior to storage, the seeds were cleaned properly and water flotation test was conducted to discard the empty and defective seeds. The experiment comprised of four storage temperatures viz., room temperature which was calculated as the average room temperature for 12 months period i.e., 19-22°C (S_1), 0+1°C (S_2), -4+1°C (S_3) and 4+1°C (S_4) and five storage containers like polythene bags (C_1), plastic jars (C_2), canvas bags (C_3), earthen pots (C_4) and tin boxes (C_5). There were thus twenty treatments each replicated thrice in a split plot design with temperature as main plot and container as sub plot. Observation on viability, moisture percent and biochemical parameters were taken at three month interval up to twelve months. The seed viability was determined by using tetrazolium chloride (TZ) test as given by Bonner (1974). The moisture content of stored seeds was determined by toluene distillation method given by International Seed Testing Association (1966) as follows:

$$\text{Moisture content (\%)} = \frac{\text{Original weight - Oven dry weight}}{\text{Original weight}} \times 100$$

The dried samples of each treatment were used to determine the biochemical parameters of the seeds. For this, one gram of dried sample was placed in 20-25 mL of boiling ethanol (80%) for 10 min and decanted. Another 10-15 mL of boiling ethanol was added to the residue. Thereafter, the two extracted samples were filtered and combined and the final volume made up to 50 mL. The alcoholic extract was then used for the estimation of total sugars. The total sugar in seed samples was estimated by phenol-sulphuric acid method given by Dubois *et al*. (1951). The soluble protein was estimated by the method prescribed by Lowry *et al*. (1951). Oil content was estimated by Peach and Tracey (1979), while the acid value was determined by the method given by Pearson (1970):

$$\text{Acid value} = \frac{56.10 \times \text{R} \times \text{Normality}}{\text{Weight of oil (g)}}$$

where, R is the amount of alkali consumed.

The refractive index was determined with the help of a Refracto-meter which gives the reading directly. The reading can be observed at 20°C either by maintaining the temperature by circulating cold or hot water through the instrument. The two year (2005 and 2006) results were subjected to Analysis of Variance (ANOVA) technique. To determine significant difference among mean values of the various treatments, we used the Duncan Test ($p \le 0.05$). Data as percentages were transformed to arcsine $(x/100)^{0.5}$.

RESULTS AND DISCUSSION

The high viability is imperative physiological criteria required of seeds that are subjected to long storage (Holmes and Buszewicz, 1958). Keeping this in mind the fresh *Pinus gerardiana* seeds were tested for their viability and biochemical status. The fresh seeds possess high viability (95.0%), moisture content (25.36%) and oil content (52.15%). It was found to be associated with low values for total sugar (4.07%), soluble proteins (13.03%), acid value (2.35) and refractive index of oil (1.413) in the seeds.

Effect of Storage Temperature

It was evident from the study that storage temperatures exert significant ($p<0.05$) influence on viability, moisture content and biochemical parameters of the seeds while the effect on oil percent and acid value was non-significant at three month storage. The data in (Fig. 1a, b) reveal that storage at 0±1°C (S_2) exhibit least decrease of viability (87.77%) after three month of storage period as compared to other treatments. This was however, followed by -4±1°C (S_3) and room temperature (S_1) giving the least viability in the seeds. The viability however, showed a steady decrease as the storage period was extended from three to 12 months. The viability of chilgoza seeds thus, decreased up to 31.57% at 0±1°C after 12 months of storage. The better performance of 0±1°C (S_2) at three month might be attributed to less loss of moisture content, biochemical parameters and oil percent in the seeds. There was an increase of total sugar (9.21%) and soluble protein (14.52%) content of the seeds up to three months after which a steady decline up to twelve month period. The seed moisture and oil content decreases continuously from 25.36 to 4.63% and 52.15 to 22.18% respectively through out the period of storage. The loss of viability at room temperature may be due to much loss of moisture content (9.40%) and oil content (50.70%) and increasing total sugar (8.07%) and soluble protein (13.43%) in seeds at three month stage. The acid value and refractive index were however, found to increase through the period of storage. Similarly, Singh *et al.* (1992) observed that maximum deteriorative

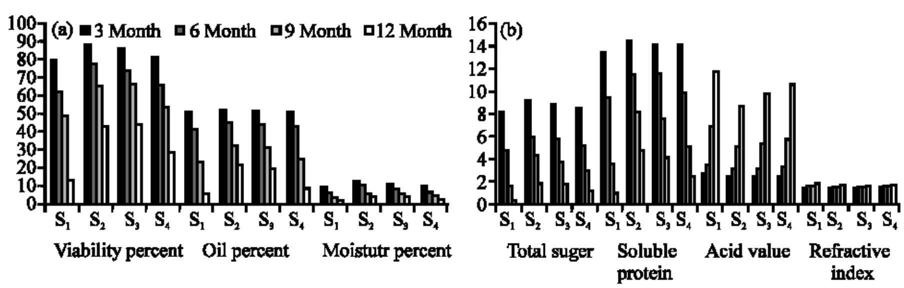

Fig. 1: (a, b) Effect of storage temperature on viability percent, moisture percent, soluble proteins, acid value and refractive index. S_1: 9-22°C, S_2: 0+1°C, S_3: 9±1°C and S_4: 4±1°C

physio-chemical changes occurred in the seeds during the latter half of storage period (June to November). The findings are thus in agreement with the work of Gordon *et al.* (1972) who reported that *Pinus merkusii* seeds respond well to storage temperature of 2°C producing a maximum germination of 80% even after three years of storage while room temperature showed significant loss of germination at 3-4 months of storage. Effendi and Sinaga (1996) on the other hand, reported that four month storage at 4°C proved the best resulting in superior germinability in the red wood. The observations also coincide with the findings of Yap and Wang (1983) in *Pinus caribaea* and *Tectona grandis,* who reported almost similar results on germinability of seeds. The increasing acid value (13.29) and refractive index (1.872) at twelve month is indicative of decreasing seed oil quality which might be attributed to breakdown of lipids and subsequent production of free radicals resulting in loss of seed viability (Koostra and Harrington, 1969). Similar findings of loss of viability at different temperatures have also been reported by Muller and Bonnet (1982) in beech seed. The other reason for the loss could be the depletion of food reserve and change in respiratory metabolism during storage (Abdul-Baki and Anderson, 1970). The findings are also in accordance with the work of Donald and Jacobs (1990) in *Pinus elliottii, P. patula, P. radiata* and *P. taeda* seeds.

Effect of Storage Containers

The viability and biochemical parameters showed significant difference ($p<0.05$) at nine and 12 month by different storage containers while 3rd and 6th month exhibit non-significant effect among different treatments. It is evident from (Fig. 2a, b) that earthen pot storage (C_4) allowed better viability and biochemical parameters in the seed. The minimum viability, however resulted when storage was done in tin boxes. There was a progressive decline in viability of seeds i.e., 90.50 to 42.29% as the storage was extended from three to twelve month in earthen pots. The better performance of earthen pots might probably be attributed to less reduction in moisture (12.19%), oil content (52.14%) and increased value of total sugar (9.62%) and soluble protein (14.94%) at three month. The earthen pot thus keeps proper balance/exchange of gases and slows down various biological processes resulting in higher viability as compared to other containers. The low viability and biochemical parameters in tin boxes on the other hand might be ascribed to more reduction of moisture (10.32%) and biochemical contents besides infiltration of harmful mycoflora and fauna in the seeds. These observations are in accordance with the findings of many other research workers who have found an initial increase in the biochemical constituents followed by a decrease in the later stages of storage in seeds of various tree species (Mallareddy and Sharma (1983) in

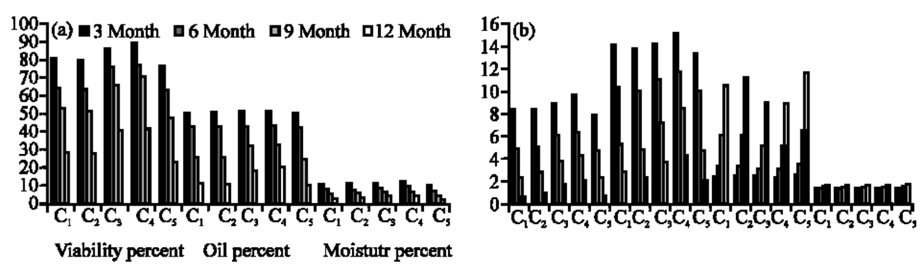

Fig. 2: (a, b) Effect of storage container on viability percent, oil percent, moisture percent, total sugar, soluble proteins, acid value and refractive index. C_1: Polythene bags, C_2: Plastic jars, C_3: Canvas bags, C_4: Earthen pots and C_5: Tin boxes

Quercus nigra; Blanchu *et al.* (1990) in Citrus seeds). The results thus, get support from the work of Bhardwaj and Gupta (1998) who reported that cloth bag storage of *Pinus gerardiana* seeds exhibit minimum percent incidence of fungi during nine month storage. The maximum spoilage of *Penicillium citrinum* and *Trichothecium roseum* was recorded on seeds stored in tin boxes, plastic jars and polythene bags. Whereas, cloth bags, gunny bags, wooden boxes and cardboard boxes remained free form any visible moldy growth even after nine months of storage. The results are also in line with those of Barton (1954) in *Pinus pseudotsuga*, *Pinus ponderosa* and *Tsuga heterophylla* and Donald and Jacobs (1990) in *Pinus elliottii*, *P. patula*, *P. radiata* and *P. taeda* seeds.

Interaction Effect

The perusal of data (Table 1, 2) reveal that interaction SxC had a significant influence ($p<0.05$) on viability and biochemical parameters in *Pinus gerardiana* seeds. It is evident that seeds stored at 0±1°C in earthen pots (S_2C_4) excelled all other treatments in viability, moisture content and biochemical parameters with minimum acid and refractive index values. This also coincides with higher total sugar (9.97%) and soluble proteins (15.96%) at three months storage. There was a progressive decline in viability of seeds i.e. 94.30 to 65.0% at 0±1°C in earthen pots (S_2C_4) followed by S_1C_5, S_2C_3, S_3C_3 and S_3C_4 for 3 months, S_2C_3 and S_3C_4 for 6 and 9 months over other storage devices as the storage was extended from three to twelve month. This was also followed by biochemical parameters especially the sugar, soluble protein and oil percent in the seeds. The seed oil percent decreased during three month storage with greater decline at room temperature than 5°C in the seeds of *Alnus firmifolia, Cedrela odorata, Swietenia microphylla* and *Cordia eleagnoides* except *Abies religiosa* reported by Carrillo *et al.* (1994). Whereas, Stolyhwo and Janson (1999) observed a small decrease in fat content of seeds over extended storage from initial 36.6% of dry mass to 33.5

Table 1: Interaction effect of storage temperature and container on viability and moisture and oil content of chilgoza seeds

Treatments	Months of storage											
	Viability (%)				Moisture (%)				Oil (%)			
(S×C)	3 m	6 m	9 m	12 m	3 m	6 m	9 m	12 m	3 m	6 m	9 m	12 m
S_1C_1	75.83 (60.68)	61.67 (51.78)	43.33 (41.17)	15.83 (23.39)	8.96	6.47	4.41	2.72	51.55	43.01	24.59	5.25
S_1C_2	75.50 (60.37)	58.33 (49.81)	45.00 (42.13)	10.00 (18.44)	9.14	5.93	4.45	3.11	49.79	40.74	25.14	5.76
S_1C_3	80.17 (63.58)	63.33 (52.75)	53.33 (46.91)	16.67 (24.05)	9.67	6.96	3.79	2.84	50.74	41.95	25.04	7.18
S_1C_4	93.33 (75.10)	64.17 (53.27)	61.67 (51.76)	19.17 (25.89)	10.67	7.68	4.83	3.02	51.71	42.92	25.14	8.28
S_1C_5	74.17 (59.56)	61.67 (51.75)	40.83 (39.71)	7.50 (15.89)	8.55	5.75	4.01	2.09	49.73	40.51	21.27	5.20
S_2C_1	87.50 (69.36)	69.17 (56.30)	56.67 (48.84)	25.00 (29.94)	11.06	7.86	6.21	3.32	50.75	43.70	26.17	13.66
S_2C_2	85.83 (67.96)	67.50 (55.27)	53.33 (46.92)	37.50 (37.76)	12.14	8.33	6.05	3.29	52.44	43.92	26.98	12.80
S_2C_3	94.17 (76.09)	91.50 (73.08)	84.17 (66.57)	28.33 (49.80)	11.84	10.20	8.00	6.54	51.03	43.08	39.60	29.13
S_2C_4	94.30 (75.91)	92.33 (73.93)	84.83 (67.86)	65.00 (53.73)	13.87	12.17	8.24	6.55	52.62	45.62	40.56	31.70
S_2C_5	79.17 (62.97)	65.83 (54.25)	50.83 (45.48)	30.83 (33.70)	10.73	8.10	5.04	2.98	51.63	44.21	25.40	12.35
S_3C_1	82.83 (65.55)	65.83 (54.29)	60.00 (50.78)	42.50 (40.69)	12.23	10.25	6.15	3.61	51.45	44.32	27.80	17.35
S_3C_2	80.67 (63.95)	62.50 (52.27)	56.67 (48.84)	37.50 (37.74)	13.72	10.15	6.50	3.70	50.73	43.71	27.22	17.31
S_3C_3	92.33 (73.95)	66.67 (68.60)	70.83 (57.43)	51.67 (45.96)	12.17	10.04	7.75	6.39	51.51	44.57	41.02	27.85
S_3C_4	92.17 (73.08)	90.00 (71.66)	81.67 (64.86)	56.67 (48.84)	13.48	11.42	7.45	6.21	52.44	45.08	39.66	32.45
S_3C_5	77.33 (61.59)	63.33 (52.74)	56.67 (48.83)	34.17 (35.73)	12.00	8.76	5.20	3.05	52.12	44.96	27.30	16.68
S_4C_1	79.17 (62.90)	63.33 (52.74)	53.33 (46.91)	30.00 (33.20)	10.83	7.63	5.41	2.33	50.95	44.22	25.36	9.31
S_4C_2	79.17 (63.01)	66.67 (54.78)	53.50 (47.01)	31.67 (34.24)	10.04	7.48	5.65	2.12	51.06	42.36	25.35	8.74
S_4C_3	81.67 (64.66)	65.83 (54.24)	56.67 (48.83)	36.67 (37.27)	9.90	6.49	5.50	2.30	50.45	42.02	24.77	9.84
S_4C_4	82.50 (65.37)	67.50 (55.26)	59.17 (50.29)	28.33 (32.07)	12.42	7.61	5.57	2.83	51.78	43.70	26.73	11.52
S_4C_5	78.33 (62.29)	65.00 (53.73)	46.67 (43.09)	19.17 (25.93)	9.98	5.80	4.30	2.14	50.10	42.05	24.51	7.04
SEm ±	1.58	1.30	1.70	1.35	0.52	0.35	0.36	0.20	0.54	0.71	0.93	1.05
$CD_{0.05}$	3.42	2.82	3.68	2.92	NS	0.75	0.78	0.43	NS	NS	1.36	2.27

Values in parentheses are arc sine transformed values. 3 m: 3 month, 6 m: 6 month, 9 m: 9 month and 12 m: 12 month

Table 2: Interaction effect of storage temperature and container on biochemical parameters of chilgoza seeds

Treatments (SxC)	Months of storage: Total sugar 3 m	6 m	9 m	12 m	Soluble proteins 3 m	6 m	9 m	12 m	Acid value 3 m	6 m	9 m	12 m	Refractive index 3 m	6 m	9 m	12 m
S_1C_1	7.67	4.31	1.42	0.13	14.13	8.75	3.26	0.42	2.58	3.58	6.65	11.33	1.463	1.510	1.654	1.806
S_1C_2	8.23	4.47	2.10	0.33	13.06	8.90	3.76	0.96	2.55	3.65	6.85	12.27	1.462	1.521	1.640	1.798
S_1C_3	8.08	5.01	1.24	0.24	13.11	9.48	3.91	1.17	2.51	3.27	6.52	11.02	1.449	1.510	1.588	1.755
S_1C_4	9.12	5.99	2.24	0.86	14.19	10.62	4.56	1.79	2.52	3.32	6.57	10.81	1.448	1.510	1.568	1.749
S_1C_5	7.26	3.81	1.23	0.11	12.69	9.25	2.75	0.30	2.80	3.91	8.03	13.29	1.476	1.531	1.720	1.872
S_2C_1	8.35	4.86	2.30	0.66	14.24	11..25	5.16	2.47	2.48	3.09	5.59	9.90	1.439	1.493	1.532	1.631
S_2C_2	8.73	5.50	2.49	0.95	14.26	11.28	5.39	3.17	2.46	3.16	5.65	10.16	1.446	1.508	1.553	1.652
S_2C_3	9.48	7.00	5.52	3.08	13.84	12.34	10.26	5.71	2.43	3.01	4.47	6.98	1.429	1.453	1.494	1.609
S_2C_4	9.97	7.22	5.81	3.13	15.96	12.99	11.78	6.22	2.39	2.96	4.32	6.93	1.436	1.455	1.495	1.556
S_2C_5	7.82	4.52	2.29	0.87	13.42	10.76	5.11	3.56	2.54	3.30	5.91	9.34	1.458	1.515	1.601	1.710
S_3C_1	8.81	5.54	3.43	1.33	13.79	11.02	6.06	3.58	2.51	3.20	5.84	10.98	1.443	1.501	1.547	1.628
S_3C_2	8.96	5.36	3.66	1.26	13.99	11.22	5.78	3.45	2.47	3.11	5.80	11.58	1.451	1.503	1.564	1.620
S_3C_3	9.63	6.64	5.28	2.25	14.95	11.90	8.96	5.47	2.43	3.09	4.44	7.33	1.433	1.470	1.526	1.580
S_3C_4	9.83	6.55	5.75	2.87	14.63	12.17	11.15	5.88	2.51	3.10	4.32	7.64	1.437	1.464	1.541	1.615
S_3C_5	8.71	5.78	3.40	1.16	13.90	10.64	8.18	5.26	2.52	3.20	6.28	11.61	1.457	1.512	1.587	1.725
S_4C_1	8.51	4.73	2.37	0.97	13.85	10.01	4.74	2.09	2.50	3.37	5.91	9.62	1.445	1.505	1.554	1.705
S_4C_2	7.58	4.85	3.25	1.50	13.62	8.86	4.13	1.67	2.50	3.27	5.92	10.40	1.453	1.510	1.563	1.727
S_4C_3	8.49	5.84	3.17	1.47	14.31	10.41	5.44	2.58	2.47	3.15	5.32	10.18	1.443	1.480	1.509	1.724
S_4C_4	9.58	5.86	3.53	1.22	14.98	10.73	6.40	3.55	2.50	3.24	5.48	10.47	1.440	1.470	1.512	1.675
S_4C_5	7.98	4.91	2.69	0.74	13.07	9.76	4.73	2.35	2.55	3.68	6.19	12.11	1.457	1.530	1.592	1.820
SEm ±	0.45	0.30	0.27	0.12	0.34	0.50	0.40	0.20	0.09	0.25	0.30	0.67	0.004	0.003	0.008	0.016
$CD_{0.05}$	NS	0.65	0.58	0.26	NS	NS	0.86	0.43	NS	NS	NS	1.45	NS	0.006	0.017	NS

Values in parentheses are arc sine transformed values. 3 m: 3 month, 6 m: 6 month, 9 m: 9 month and 12 m: 12 month. S_1: 9-22°C, S_2: 0±1°C, S_3: -4±1°C and S_4: 4±1°C. C_1: Polythene bags, C_2: Plastic jars, C_3: Canvas bags, C_4: Earthen pots and C_5: Tin boxes

and 32.5%, respectively with germination capacity of 90% in freshly collected seeds to 70 and 30% after 15 to 20 years of seed storage in Norway spruce. The results also get support from Singh *et al.* (1992) who studied the physio-chemical changes in chilgoza seeds when stored in finely webbed gunny bags under ambient laboratory conditions and reported that biochemical contents were considerably reduced (total sugar, proteins and oil content) in seeds during storage. This might also be attributed to the fact that a relatively better biological process or less physiological deterioration of biochemical was caused as compared to other treatment combination. This obviously resulted in maintaining/enhancing the seed longevity in the species. The least values of above parameters found in treatment combination S_1C_5 (Room temperature x tin boxes) might be ascribed to faster physiological deterioration which therefore reduced the germinability performance in the seeds. This might also be due to wide temperature flucation, the high relative humidity and high activity of mycoflora and fauna present in seeds which start feeding on endosperm rendering the seeds non-viable. The results are in agreement with the findings of Gupta and Raturi (1975), who conducted viability tests in a number of forest tree seeds. The findings are in harmony with Barton (1954) in *Pinus ponderosa*, *Pseudotsuga menziessii* and *Tsuga heterophylla*, Donald and Jacobs (1990) in Pinus species and Stolyhwo and Jason (1999) in Norway spruce.

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