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Seed storage behaviour of *Buxus wallichiana* Baillon: An important woodcraft species of Indian Himalayan region

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Abstract
Seeds of *Buxus wallichiana* Baillon are dormant and not much information is available on their shelf life under storage conditions. Therefore, this study was conducted to investigate the seed storage behaviour of *B. wallichiana*, an important woodcraft species of the Indian Himalayan Region. Healthy matured fruits of *B. wallichiana* were collected from Matkangra block, Chakrata Forest Division of Uttarakhand. The seeds were extracted, cleaned, processed manually and their initial viability was tested through germination test. After initial viability determination, seeds were processed and placed in air-tight plastic boxes for storage at four temperatures *viz.,* ambient room temperature (Control), 5°C, 15° C and -20°C. Thereafter, at every two months interval, germination test was conducted to assess the viability of the stored seeds. The results revealed that the initial viability of freshly collected seeds was 26.66 per cent and it took 7.83 days to complete germination. After two months of storage the germination percentage increased in the seeds stored at all the temperatures going up to maximum (73.33%) in the ones stored at -20°C followed by 15°C (66.67%) as compared to 50% in the seeds stored at room temperature. Similarly, the mean germination time also increased proportionally, longest being 44 days for seeds stored at -20°C. Seeds stored at ambient room temperature lost viability completely at 6 months storage duration while almost half the viability was lost in seeds stored at 15°C (36.67%) and -20°C (26.67%). From present study, it can be concluded that the longevity of the *B. wallichiana* seeds is around 12 months when the seeds are stored at lower temperatures, maintaining the viability about 23% at 5°C and 15°C and 16.67 % at -20°C.

Keyword: Buxus, Germination, Seed storage, Temperature, Viability

INTRODUCTION

The Himalayan boxwood (*Buxus wallichiana* Baillon) is a dense leafy evergreen shrub or small tree found commonly in the western and central Himalayas at elevations ranging from 1200 m to 2900 m amsl, where it grows gregariously in patches in moist shady ravines. The wood is used to manufacture the precision instruments, boxes, turnery works, etc., while extracts of the leathery leaves and bark is used in local medicine (Govt. of India, 1986). But the species is facing severe threats in many parts of the Himalayan region because of its over exploitation for woodcrafts industry (Pant, 2011). Since, *B. wallichiana* has been identified as a potential species for reforestation in the denuded hill tops in higher elevations of upper and middle Himalayas, understanding the natural dispersal mechanisms, seed ecology and seed storage and survival processes can help to develop strategies for natural and artificial regeneration programmes (Viswanath *et al.* 2006). However, there is limited information on the germination requirements and seed storage behaviour of boxwood seeds. Thapliyal (1992) and Viswanath, (1999) have reported that the seeds of *B. wallichiana* are difficult to germinate in laboratory as well as in the nursery. Shirzad *et al.* (2013) reported reduction in the number of stands of *B. hyrcana* in Caspian forests due to its exploitation for wood. The seedling growth of other species like *B. microphylla* is reported to be irregular owing to erratic germination pattern. So, the seed storage study is very essential for determining shelf life and viability of various types of seeds for conservation and regeneration of species of high conservation concern. Good quality seed must be collected and should be stored properly according to the seed storage procedures to prevent loss of quality of

seeds. Thus, the present study was undertaken to investigate the longevity of seeds of *B. wallichiana* at various storage temperatures and to develop a strategy for conserving the species *ex-situ.*

MATERIALS AND METHODS

Site and seed collection: Present study was conducted at the Forest Tree Seed Laboratory of Forest Research Institute, Dehradun. The seeds of *B. wallichiana* were collected in the month of September from Matkangra block near Jadi village of Chakrata Forest Division, Uttarakhand, India having Geo-coordinates 30º44.069'N and 77º50.621'E and altitude 2162 m amsl.

Initial parameters of the seed: After collection the initial parameters of fruits (colour, length, width, 100 fruit weight) and seeds (colour, length, width, 100 seed weight, purity, moisture content, and germination percentage) were studied. Fruit and seed colour was observed visually. Fruit and seed length was measured using digital calliper. The observations on weight of 100 fruits and seeds was taken in three replicates (ISTA, 2010). The purity percentage of the seeds was calculated after seed extraction using the formula:

Purity % = Weight of pure seed sample/Weight of total working sample×100 …Eq.1

The moisture content (on fresh weight basis) of collected seeds was determined in three replications of 10 seeds each in oven at 103±1º C (ISTA, 2010) for 17±1 hours using the following formula:

Moisture content %= Fresh weight- Oven dry weight/ Fresh weight×100 …Eq. 2

The germination trial was conducted in laboratory by placing four replications of 10 seeds each on moist Whatman filter paper in a petri dish (14 cm diameter) and kept in a germinator at 25±1ºC. The seeds were considered as germinated when the radicle emerged about 0.5 cm, thereby, germination per cent was calculated.

Germination %= Number of germinated seeds/ Total seed sown ×100 …Eq. 3

Germination value: Germination value is an index combining speed and completeness of seed germination.

Germination value (GV) was calculated by the method given by Czabator (1962).

Germination value (GV) = Final DSG * PV DSG … Eq. 4

Where DSG = Daily Germination Speed

PV = Peak Value of DSG …Eq. 5

Peak value is the highest value of Mean Daily Germination (MDG)/DSG.

DSG/MDG is calculated by dividing the cumulative germination percentage by the number of days since sowing.

Mean germination time (MGT): It is the time taken to complete germination under various treatments and was determined as Mean Germination Time (MGT) in days as under:

MGT = ∑(daily germination × days)/ Number of seeds sown …Eq.6

Seed storage experiment: The well cleaned, processed and initial viability determined seeds were desiccated to about 11% moisture content and then kept for storage at four temperatures viz. Ambient room temperature (Control), 5°C, 15°C and -20°C. Seeds were stored in plastic boxes at each temperature, in the month of November. The viability test of stored seeds was done at two months interval through germination test.

Statistical analysis: There were four treatments of storage temperatures T0 = ambient room temperature (Control), T1 =5°C, T2 =15°C and T3 = -20°C) replicated four times. The data pertaining to the present study was analyzed using Complete Randomized Design and was analyzed by using software MS Excel (2007) and SPSS version 21.

RESULTS

The fruits of *B. wallichiana* were collected when they turned brownish in colour. The length and width of fruits was 8.98±0.5mm and 8.39±0.47mm, while the weight of fruits (100 individuals) was 39.33 ± 0.58 g. The seeds were extracted manually, which were shiny black in colour. The seed length and seed width were recorded as 5.95±0.46 mm and 2.91±0.18 mm. The initial moisture content of freshly extracted seeds was 20.86%. 100 seed weight was recorded as 1.34±0.45 g, while the seed purity was recorded as 93.98% (Table 1).

The results revealed that the initial viability of the seeds was 26.66% and it took 7.83 days to complete the germination with germination value of 0.65. After two months of storage the germination increased in all the storage temperatures going up to 73.33% in -20°C followed by 63.67% germination in 15°C as compared to 50% germination in seeds stored at ambient room temperature (Table 2). However, mean germination time also increased proportionally, longest being 44 days for seeds stored at -20°C with 0.59 germination value. Similarly, after two months of storage the Germination Value (GV) of seeds also increased from initial GV (0.65) to maximum in 1.04 in 15°C as compared to seeds in control (0.71) while it was minimum (0.54) in the seeds stored at 5°C. After 4 months in storage, germination percentage increased to 60% in seeds stored at 5°C while, it reduced marginally in the ones stored at 15°C and -20°C, but more than 50% loss in viability was observed in seeds stored at RT (Table 3). Seeds stored at ambient temperature lost complete viability at 6 months while almost half the viability was lost in seeds stored at 15°C and -20°C, loss in germination value of seeds was also recorded due to loss in viability. Viability of seeds stored at all the three temperatures further dropped significantly (p≤ 0.05) at 8 months and further at 10

Table 1. Fruit and seed parameters of *B. wallichiana.*

S. N.	Parameter	Fruit	Seed
1.	Colour	Brown	Black
2.	Length (mm)	8.98±0.5	5.95±0.46
3.	Width (mm)	8.39±0.47	2.91±0.18
4.	Moisture Content %	--	20.86±6.85
5.	Weight (g) 100 individuals	39.33±0.58	1.34±0.45
6.	Purity %	--	93.98± 2.26
7.	Germination %	--	26.66± 5.77

Mean±SD: 100 individuals

Table 2. Effect of storage temperature on mean germination of seeds of *B. wallichiana.*

Months	Storage Temperatures			
	RT	5^0C	15^0C	-20^0C
0	**26.66± 5.77**	**26.66± 5.77**	**26.66± 5.77**	**26.66± 5.77**
2	50 (45.0)	53.33 (46.92)	66.67 (54.78)	73.33 (59.71)
4	23.33 (28.77)	60 (50.94)	63.33 (52.77)	70 (57.70)
6	0.0	40 (39.15)	36.67 (33.89)	26.67 (30.99)
8	0.0	30 (33.29)	30 (33.0)	20 (26.07)
10	0.0	26.67 (31.0)	20 (26.56)	16.67 (23.85)
12	0.0	23.33 (28.29)	23.33 (28.77)	16.67 (23.85)
SEm		2.086		
LSD @5%		4.172		

Values in parentheses are arcsine transformed

Table 3. Effect of storage temperature on germination value and mean germination time of seeds of *B. wallichiana.*

Months	GV				MGT (days)			
	RT	5^0C	15^0C	-20^0C	RT	5^0C	15^0C	-20^0C
0	0.65	0.65	0.65	0.65	7.83	7.83	7.83	7.83
2	0.71	0.54	1.04	0.59	23.17	25.70	32.13	44.37
4	0.10	0.58	0.79	0.88	12.13	33.70	26.53	37.40
6	-	0.30	0.50	0.30	-	19.47	16.83	11.33
8	-	0.19	0.15	0.10	-	14.37	15.50	9.70
10	-	0.21	0.07	0.06	-	11.17	9.53	9.23
12	-	0.18	0.11	0.07	-	9.87	4.53	8.83

months duration while MGT also decreased as seeds took less time for germination. Germination of seeds stored at 15°C increased from 20% to 23% at 12 months, this increase however was not significant. Seeds during storage got damaged and developed emptiness due to which the experiment could not be continued beyond 12 months. Longevity of seeds was assessed to be around 12 months and 5, 15 and -20°C were better suited storage temperature for longer viability of seeds.

DISCUSSION

The freshly collected seeds of *B. wallichiana* exhibited low viability (26 %) which increased up to 73%, in the seeds stored at -20^{0}C followed by 67% viability at 15^{0}C as compared to 50% in control (ambient room temperature) after two months in storage. The results observed might be due to the presence of shallow physiological dormancy in the seeds of *B. wallichiana* which may have broken down after getting prechilling treatment under low temperature storage conditions. In order to break the seed dormancy and get them germinated moist stratification for 30 days has been recommended for the seeds of *B. wallichiana* by Thapliyal *et al.* (2017). Similarly, Lazaro *et al.* (2006) reported low seed germination in the five tested populations of *Buxus balearica* in the germination chamber where they recorded the maximum germination for the seeds collected from site Ragol I (41%) while only 9% in the seeds collected from site Galatzo.

Similarly, very poor or no germination of *B. sempervirens* was reported by Cetin *et al.* (2015), while working on provenance variation of the species. They have reported only the seeds collected from Duzce province had about 1 % germination rate at 15^{o}C constant temperature while seeds from other provinces did not yield any germination. After four months of storage the viability of stored seeds declined gradually in all the storage conditions while rapidly in the seeds stored at ambient room temperature (23%) (Table 2). The seeds of *B. wallichiana* exhibited the problem of emptiness (up to 50%), during storage the seeds got spoilt (turned empty) due to which their longevity was observed to be about a year when stored at 5, 15^{0}C and sub zero temperature with seeds maintaining 26, 13 and 16% viability, respectively, while no germination was recorded after four months of storage at ambient room temperature. It has been reported in other species of *Buxus* like *B. microphylla* that

seeds cannot be dried and stored for long, dried seed germination rate was low and seeds did not germinate for a long period of time Likewise, Viswanath *et al.* (2006) observed that a high fraction of seeds of *B. wallichiana* do not remain viable for more than four to five months. This may be the reason why *B. wallichiana* seed bank dynamics appears to follow a transient strategy rather than a persistent seed bank strategy in nature.

Conclusion

Seed storage studies are very important for determining shelf life and viability of various types of seeds and devising strategy for their *ex-situ* conservation of the diversity of species through seeds. The longevity of the *B. wallichiana* seeds in the reported study was observed to be around 12 months when the seeds were stored at low temperatures maintaining the viability about 23% at 5°C and 15°C. Although this investigation does provide preliminary evidences about longevity of the seeds of *B. wallichiana* but still there is a scope for more such studies on seeds of *B. wallichiana* from different provenances, to devise best strategy for conservation of the species.

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