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Effect of Short-term Storage Conditions on Seed Germination of *Fraxinus micrantha*

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ABSTRACT: *Fraxinus micrantha,* a multipurpose tree species in the Indian Himalyan Region (IHR) has reportedly poor regeneration status and high mortality in the early stage of seedling development that causes vulnerability for plant existence in the natural geographical locations. The aim of this study was to investigate the storage potential of *F. micrantha* and to formulate the methodology for *in situ* conservation of the seed germplasm of the species. The laboratory experiments have investigated the germination response of *Fraxinus micrantha* seeds to short storage conditions in hermetically sealed containers at 6% moisture content at 5ºC. Initial germination of the seeds was recorded as 90%. The viability of the stored samaras was tested quarterly upto a period of 28 months. Germination parameters such as Mean Germination Time, Germination value, Peak value and Germination Index of the species were measured as per the standard methods. Mean Germination Percent (GP) of stored seed varied between 70% and 95%. Mean germination time of stored seeds ranged between 5.4 to 8.9 days. Germination Percent, Mean Germination Time and Germination Value under different storage period varied significantly (P<0.05). The results indicate that seeds of *F. micrantha* maintained high germinability during short-term storage conditions thus exhibiting good storage potential for conservation of the genetic resources of the species as seed germplasm in gene bank conditions, for posterity.

Keywords: conservation, germination, mean germination time, germination value, short-term seed storage.

INTRODUCTION

Seeds are the primary propagules for regeneration and reintroduction of plant species in ecological restoration to mitigate environmental degradation and species extinction (Broadhurst *et al.,* 2008; León-Lobos *et al.,* 2012; Elzenga and Bekker, 2017). Most of the wild trees produce seed in abundance but the *in- situ* regeneration does not occur in that proportion. *In-situ* preservation, species reintroduction, restoration and afforestation programmes require high quality and genetically diverse seed germplasm, but limited supply of seed in many species and non availability of quality seed are major constraint (Thapliyal *et al.,* 2018). A regular inventorization and monitoring of plant diversity is required to assess the present status of plant diversity and making suitable conservation strategies for future (Chandra *et al.,* 2021). Naturally emerged seedlings of *Fraxinus micrantha* showed 90 % mortality within a few months (Malik and Bhatt, 2016) resulting in poor regeneration status of the species that causes high risk for its future existence in the forest ecosystem. Thus for achieving long term sustainability of species which are at edge towards threatened level require special conservation strategy through *ex-situ* and *in-situ* methods.

Fraxinus belongs to the family Oleaceae which comprises about 45 tree species. Its fruits are samara type, characterised by long terminal wing. The fruit of *Fraxinus* has two ovules per locule but usually only one ovule develops, making the samara one-seeded and are wind pollinated (Wallander and Albert, 2000). Fraxinus species are mainly distributed in temperate and subtropical regions of the Northern Hemisphere (Wallander and Albert, 2000). In India, *Fraxinus* species are mainly distributed in temperate Himalayas. *Fraxinus micrantha* (Uttarakhand), *F. xanthoxyloides* (Kashmir and Uttarakhand), *F. floribunda* (Himalayan region of Uttarakhand, eastern Himalayas and Khasi hills of Meghalaya and Manipur), *Fraxinus griffithii* (Mishmee Hills in Arunachal Pradesh), *Fraxinus excelsior* (Kashmir, Ladak), *Fraxinus hookeri* (Jammu & Kashmir) and *Fraxinus suaveolens* (Sikkim, West Bengal) are some of the species reported in India (Kritikar and Basu, 1988; Green, 2003). *Fraxinus uhdei* is one of the exotic species introduced in India (Shukla and Sangal, 1980).

F. micrantha is found associated with *Quercus floribunda, Q. leucotrichophora, Abies pindrow, Aesculus indica, Juglans regia, Acer caesium*, etc. Timber of this species is used in manufacturing sports articles, furniture, in construction, implements, etc.

Several species of *Fraxinus* are used in the traditional medicine for the treatment of various diseases. *F. micrantha* is traditionally used in the treatment of liver disorders (Singh *et al.*, 2019). Biochemical analysis in the aerial parts of *F. micrantha* reported the presence of flavonoids, polyphenols, and sterols which possess potent anti-proliferative activity on the malignant breast carcinoma cell line (Kumar and Kashyap, 2015).

The conservation programmes through *ex situ* seed storage are crucial for maintaining the vigour and viability of seeds for longer period. Seeds can store from few months to years depending on the species and the storage conditions. Information on seed storage behaviour is available only for about 3% of higher plants (Phartyal *et al.*, 2002). Seeds are categorised into microbiotic (seeds with life spans not exceeding 3 years); mesobiotic (seeds with life spans from 3 to 15 years); macrobiotic (seeds with life spans from 15 to 100 or more years) based on the life span under ordinary storage conditions (Ewart, 1908). Seeds of many temperate species like *Acer*, *Carpinus*, *Crataegus*, *Euonymus*, *Fraxinus*, *Liriodendron*, *Nothofagus*, *Prunus*, *Pyrus*, *Sorbus* and *Tilia* can be stored moist at low temperatures of 3-5°C for short periods (Holmes & Buszewicz, 1958). However the stored seeds are monitored for viability during storage conditions which must balance timely detection of loss in viability.

Complete data on long term storage of *Fraxinus* species are only available for few species like *Fraxinus pennsylvanica* and *F. excelsior*. The present study investigated the effect of short-term seed storage condition on the longevity of the seeds of *Fraxinus micrantha* which further helps to develop systematic long term seed germplasm conservation strategies in *ex situ* without affecting the inherent seed vitality and storage quality.

MATERIALS AND METHODS

A. Materials

Samaras of *F. micrantha* were collected from Dhanaulti, Mussoorie Forest Division (Geo-coordinates 30° 44′ 38.4″N and 78° 20′ 44.64″E) in Uttarakhand state of India, in September 2017. The samaras were packed in bags and transported to the laboratory. The fresh seeds were processed and cleaned and thereafter they were evaluated for initial germination and their moisture content was determined by low constant temperature oven drying method (ISTA, 2010). Moisture content of the seeds was determined in hot air oven at 103°C temperature for 17 hours. Viability and other germination parameters of the fresh seeds initially were evaluated through germination tests that were conducted every three months to evaluate possible changes in seed quality. Seeds were randomly sampled for all the experiments. Four replications of 25 seeds each were used for the germination experiment in a seed germinator at a temperature 25°C±1°C. Seeds were plated on moist Whatman filter paper No.1 in petri-plates. The paper was moistened with double distilled water as and when deemed necessary to avoid the substratum from drying. As the pericarp of samaras of *F. micrantha* are reported to inhibit seed germination (Thapliyal and Nautiyal, 1989) the seeds were plated for germination after complete removal of the pericarp. Germination parameters such as germination percent (ISTA, 2010); Mean Germination Time (Orchard, 1977); Germination value (GV) which reflects speed and completeness of germination, was determined at each treatment, was determined according to the equation given by Djavanshir and Pourbeik (1976); Peak value (Czabator, 1962), and Germination Index (Timson, 1965) of the species were measured as follows:

Germination percentage (GP) = total number of seeds germinated at end of germination test/total number of seeds plated for germination test.

Mean germination time (MGT) = ΣFx/ΣF; where F is the number of seeds germinated on day x.

Timson germination index (TGI) = ΣG/T, where G is the percentage of seed germinated per day, and T is the germination period.

Germination value was expressed as ∑DGS/N x (Final cumulative Germination Percent/10); where DGS is daily germination speed which is calculated by dividing cumulative germination percent by the number of days since beginning the test, N is number of counts and 10 is constant through germination test.

F. micrantha seeds were desiccated to 6% moisture content in cool-air seed dryer (15% RH and 15°C) and thereafter placed in hermetically sealed boxes at 5°C ±1°C temperature in storage chamber in short-term storage condition up to a period of 28 months. The germination test on a random sample of the stored seeds was carried out after every three months to evaluate the changes in seed viability.

B. Statistical analysis

Statistical analysis of germination data was performed with the SPSS 16.0 software package. The data were subjected to analysis of variance (ANOVA), Post hoc tukey test was used to check the significance of storage period on GP, MGT and GV.

RESULTS AND DISCUSSION

A. Effect of storage period on Germination behaviour of Fraxinus micrantha

Germination of *F. micrantha* seed was observed up to a period of 28 months under storage conditions. Initial mean germination of samaras at 6% moisture content was observed as 90%. Highest and lowest mean GP within this period was observed as 95% (at 25 months of storage) and 70% (28 months), respectively (Fig.1). Mean GP above 90% was observed after one, seven, ten, thirteen and twenty five months of storage. Increase in germination can be attributed to the dry chilling treatment to the seeds would have got during the storage period.

Mean germination time of stored seed ranged between 5.4 to 8.9 days. Lowest mean germination time 5.4, 5.5 and 6.2 days were observed after 10th, 25th and 1st month of storage, respectively which means that the seed germination was faster and completed in less time. Highest mean germination time 8.9 days were observed after 13th month of storage. An increasing and decreasing tendency in the mean germination time (in days) was observed in the stored seeds (Fig. 1).

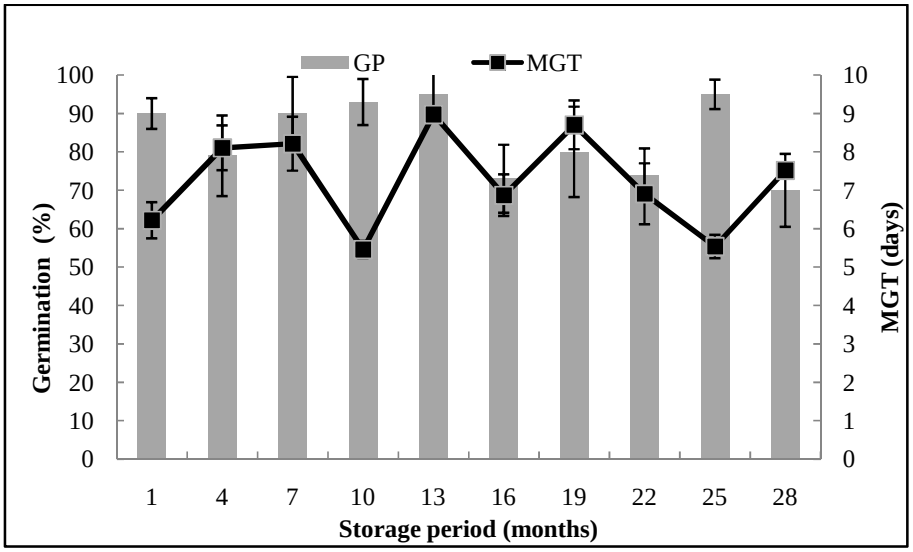

Fig. 1. Effect of storage period on germination percent and mean germination time in seeds of *F. micrantha.* Vertical error bars represent the standard deviation.

B. Effect of storage period on Germination value, Peak value and Germination Index

Germination value of stored seeds of *F. micrantha* ranged between 18.3 to 59.2. Peak value ranged between 6.3 to 12.2 and germination index ranged between 2.0 to 3.7. All the parameters were highest in 25th month followed by 10th month and 1st month (Fig. 2).

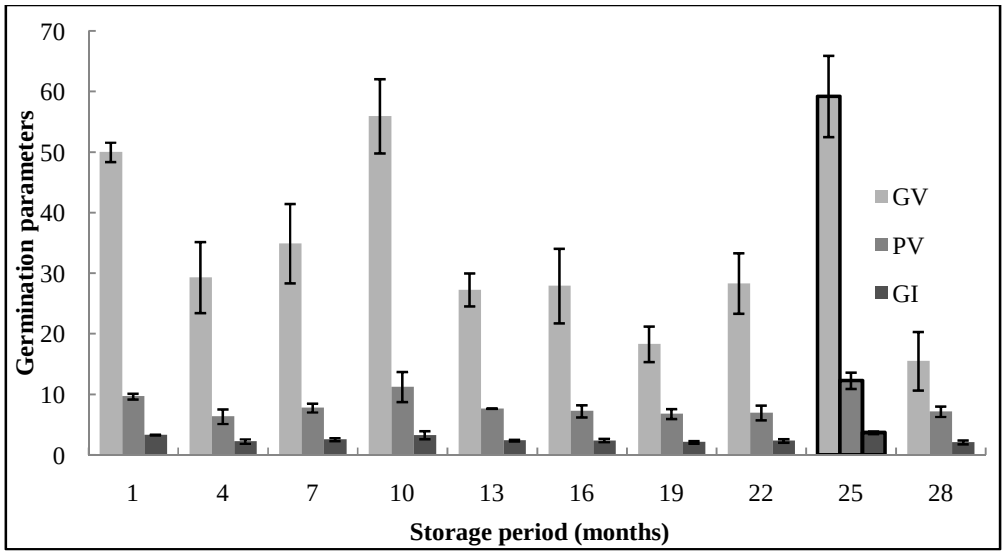

Fig. 2. Effect of Storage period on GV, PV, GI on seeds of *F. micrantha*. Vertical error bars represent the standard deviation.

Statistical analysis of the data shows significant differences between group means as determined by one-way ANOVA. ANOVA showed a significant difference (P<0.05) on GP, MGT and GV under different storage period (Table 1). Post hoc tukey test exhibited highest germination percent in 13th and 25th month of storage period which are significantly different (P<0.05) from other storage period. Similarly lowest MGT was exhibited in 10th and 25th month of storage period. Highest range of GV of seeds was observed at 1st, 10th and 25th month of storage period and significantly different (P<0.05) from that other testing durations. Several studies have reported a close relationship between seed viability and storage period (Ellis and Roberts, 1981, Ellis *et al.*, 1991). Seed moisture content, temperature and storage periods are among the main factors affecting above relationship (Roberts, 1973). Long-term storage studies with seeds of the ashes though are few but these have confirmed orthodox nature of seed of ashes in their storage characteristics (USDA, 2008) which is also evident by the findings of the current investigation. Studies by Barton (1945) reported no loss in viability for 7 years for *Fraxinus pennsylvanica* and *F. excelsior* seeds stored in sealed containers at 5°C with seed moisture content of 7-10%. Similar conditions have proved successful for flowering ash (Heit, 1967) and Shamel ash (Bonner, 1974). Germination capacity of *Fraxinus*

pennsylvanica Marsh. (green ash) seeds remained high after 1 year of storage at 0°, 24 to 35° F, but only for the 0°F (-17°C) storage after 6 and 8 years (Cram, 1984). The effects of drying and storage on the quality of *F. angustifolia* pretreated seeds subjected to 2 weeks of warm stratification + 2 weeks of cold stratification were investigated by Piotto (1997). After 4, 8 and 12 months of storage at -3°C, significant differences were observed in the germination rate. Tilki and Cicek (2005) reported slowing down of germination of stored seeds of *Fraxinus angustifolia* subspp. *oxycarpa* after 12 months of storage at +4°C. They also reported that seeds can be stored successfully for 12 months at -5°C after drying to 11 ±1%.

Table 1: Effect of storage period on GP, MGT and GV of *Fraxinus micrantha* seeds.

Storage period (months)	GP	MGT	GV
1	90 (2.00)^{bcd}	6.21(0.229)^{ab}	49.5(0.645)^{d}
4	79(5.25)^{abcd}	8.10(0.296)^{def}	29(2.972)^{bc}
7	90(4.76)^{bcd}	8.21(0.344)^{ef}	34.5(3.379)^{c}
10	93 (3.00)^{cd}	5.45(0.111)^{a}	55.75(3.119)^{d}
13	95 (3.00)^{d}	8.97(0.108)^{f}	26.75(1.315)^{abc}
16	73(4.43)^{ab}	6.87(0.25)^{bc}	27.25(3.038)^{abc}
19	80 (5.88)^{abcd}	8.70(0.335)^{ef}	18(1.472)^{ab}
22	74 (3.46)^{abc}	6.91(0.399)^{bcd}	28(2.38)^{bc}
25	95(1.91)^{d}	5.53(0.165)^{a}	58.75(3.301)^{d}
28	70 (4.76)^{a}	7.52(0.041)^{cde}	15(2.449)^{a}
F	**5.743**	**24.613**	**35.151**
P	**<0.05**	**<0.05**	**<0.05**

Values in parentheses represent Mean Standard Error. The values with different superscript letters in a column are significantly different ($p<0.05$)

Initial germination percent of *F. micrantha* seeds was 90%. GP of stored seed increased as compared to initial GP at several times. Storage after 1st, 7th, 10th, 13th, 25th month shows an ascending pattern of germination percent in the stored seed followed by a slight descending pattern of seed germination. Rhythmic pattern of seed germination in the species was not found seasonal but progressive on both directions for certain period. GV, PV, and GI of stored seed were comparatively higher during highest germination period. MGT did not show a consistent pattern of increase or decrease so it cannot be considered coinciding with GP. Several authors have reported rhythmic pattern of seed germination in stored seeds. Garwood, (1982) reported seasonal rhythm of seed germination in a semi-deciduous tropical forest species. Rawat and Thapliyal (2003) reported seasonal endogenous rhythm in seed germination of *Dendrocalamus strictus*.

An increase in longevity with decrease in storage temperature and seed moisture has been reported in tropical highlands species of *Ulmus wallichiana* in medium term storage conditions (Phartyal *et al.*, 2003). Zasada and Densmore (1980) reported a reduction in germination after two years of sealed storage at -10°C which was less than 6.5 % of initial germination in the temperate species of *Populus balsamifera* and *Salix glauca*. Hong and Ellis (1996) recommended cold moist storage methods for short-term storage period in dormant seeds of trees and shrubs. Seeds of the genus *Fagus, Fraxinus, Liriodendron, Magnolia* and *Prunus* can be stored at low temperatures of 3-5°C for short term in moist storage (Schopmeyer, 1974; Young and Young 1992).

CONCLUSION

In the present study seed of *Fraxinus micrantha* stored for short-term storage period were tested at every quarter of the year to analyze the germination behaviour and germination parameters. Study concludes that, short-term seed storage at 6% moisture content at 5ºC ± 1ºC storage temperature did not have significant reduction in viability in terms of GP, MGT, and GV while a substantial positive pattern in germination parameters were observed from fresh seeds to stored seeds, many times.

The seeds of *F. micrantha* exhibited good storage potential for conservation of the genetic resources of the species for short to medium-term. The study concluded that seed monitoring intervals in *ex situ* seed germplasm conservation of *F. micrantha* can also be extended to six months interval (desiccated 6% moisture content, 5ºC ± 1ºC temperature) during short-term storage which can also reduce the monitoring time and human resources required for it. The seeds of *Fraxinus micrantha* exhibited good storage potential by retaining the viability for longer period in hermetically sealed containers in optimum storage conditions.

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