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Seed storage physiology of himalayan elm (*Ulmus wallichiana*): an endangered tree species of tropical highlands

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Summary

To understand the seed storage physiology of *U. wallichiana,* mature seeds were stored at different combinations of seed moisture content (10.46 and 3.09%), storage temperature (-5, 5, 15°C and RT i.e. ambient room temperature) and storage container (open and air tight sealed). Storability was quantified by different viability and vigour test methods up to 678 days of storage. The half viability period (p_{50}) which quantifies the relationship between viability, storage temperature and seed moisture content exhibited maximum p_{50} of 998 days when seeds were desiccated to 3.09% moisture content and stored at -5°C in air tight container. In contrast a minimum p_{50} of 34 days was recorded when seed stored with original moisture content of 10.46% at RT. The vigor of seed during storage quantified by mean germination time (MGT), electrical conductivity of seed leachates (EC), rate of deterioration (d^{-1}) and germination index (GI) further revealed that the consideration of -5°C and 3.09% seed moisture content was the best for long term storage. The study reveals *U. wallichiana* seeds are short lived if stored in open containers under ambient (room) conditions and the original moisture content. The seeds, can however be stored for a comparatively longer period at RT, provided they are properly dried to approximate 3.0% moisture content and stored in airtight containers. From the overall response of storage behavior of *U. wallichiana* it may be conclude that this species is possess orthodox storage physiology.

Introduction

At a current rate of deforestation in tropical regions, mass extinction of some of the species seems imminent leading to loss of considerable amount of genetic diversity within species. *In-situ* conservation of species in tropical ecosystems, particularly like those of India, is impracticable owing to factors such as environmental disasters, landslides, construction activities, forest fires, illicit felling, habitat destruction, climate changes, forest fragmentation and overexploitation of wild genetic resources. The only ideal strategy to halt the alarming rate of deforestation, loss of species and their well-adapted provenances is *ex-situ* conservation through seed storage under controlled conditions. According to Harrington (1970, 1972) of all the *ex-situ* conservation strategies, the easiest and least expensive method of preserving world's existing plant genotypes would appear to be seed storage. At present, however, information on seed storage behaviour (i.e., survival and longevity of seed under various storage conditions) is available for only about 3% of higher plant (Hong and Ellis, 1996). The optimum storage conditions must be known in order to preserve the seed under the best conditions possible while keeping costs to a minimum (FAO/IBPGR, 1992). The major consideration for seed storage studies of a given species is the determination of orthodox versus recalcitrant behaviour. In the genus *Ulmus,* species such as *U. crassifolia* and *U. serotina* mature in the fall and are orthodox in nature whereas the spring seeding *Ulmus* are weakly recalcitrant (Bonner, 1984; cited in Willan, 1985). The "Kew Seed Information Database" (Tweddle *et al.,* 2002) cites storage behaviour of 16 species of genus *Ulmus* with orthodox, probable orthodox and uncertain, unfortunately there is no maintain of *U. wallichiana* species. The present work was therefore taken up an attempt to determine storage physiology and most appropriate storage conditions for the seeds of *Ulmus wallichiana* Planchon, an endangered species of the central and western Himalayas (IUCN, 1978).

Materials and methods

Mature samaras of *U. wallichiana* were collected in May-June from Old Manali village at 2600 m in the Kullu-Manali Forest Division of Himachal State of India. Samara were manually de-winged, cleaned and upgraded by separating empty seeds from the filled ones (Phartyal *et al.,* 2002). Initial moisture content and viability of seed was determined and then slowly dried from original 10.46 to 3.09% moisture content in a low temperature dryer at 15°C/15% RH. Both dried seeds and seed with original moisture content were stored at 15, 5, -5°C and RT in sealed containers. Seed at the original moisture content were also stored in open containers at RT as control. Before storage, initial viability of seed was determined by the laboratory germination test, while the seed vigour was determined by measuring electrical conductivity of seed leachates (Bonner, 1991) and by calculating MGT using the laboratory germination data according to Bonner (1983) and germination index (an index combining per cent germination and seedling length after 14 days of seed sowing) as described by Vertucci *et al.* (1994). Viability during storage was monitored once a month for the initial four tests but once in two months afterwards. The viability and vigour of seed stored at different combinations of moisture content and storage temperature was compared for significance using ANOVA after arcsine transformation of percentage values. Probit values of germination percentage were regressed against storage time and the time taken for viability to decline to 50% of the original i.e., p_{50} according to Roberts (1973). The slope of survival curve indicated the rate of deterioration (Vertucci *et al.,* 1994). In each experiment three replications were used with different number of seeds (100 seeds for germination test, 25 seeds for EC test and 2 g seeds for moisture content test).

Results

The seed stored at the original moisture content (10.46%) in an open container at RT as control, lost viability completely within two months. The data pertaining to the effect of the different moisture contents, storage temperatures and storage periods on deterioration of seed viability (germination) and vigour (MGT) showed significant variation among themselves and their interactions (p=0.01). Seed with reduced moisture content of 3.09% showed significantly higher mean germination of 61.57% as compared to 55.40% germination recorded at 10.46% moisture content (table not presented). With increase in storage temperature, the viability of *U. wallichiana* seed declined. Maximum mean germination of 80.62% was recorded at -5°C followed by 75.05, 62.90 and 15.38% mean germination at 5, 15°C and RT respectively. The trend for both germination and MGT highlighted -5°C as best storage temperature. The interaction of seed moisture content and storage temperatures further revealed that maximum mean germination of 81.81% was recorded for seed with 3.09% moisture content stored at -5°C, while significantly minimum mean germination of 11.62% was observed for seed stored at RT with 10.46% moisture content. Seed stored at this combination also took more time (13.86 days) to complete germination as compared to other combinations.

The seed stored with 10.46% moisture content lost viability/germinability completely within 92 days at RT but took 189 days at the same temperature when dried to 3.09% moisture content. In contrast, seed stored at lower temperatures retained some viability even after 678 days irrespective of seed moisture content but the percentage of viable seed varied among temperatures and moisture content used for storage (figure 1). The seed stored at 5, -5°C and original moisture content retained viability with 44 and 57.33% germination and 58.67 and 70.67% with reduced moisture content, respectively when tested after 678 days of storage. All these values were significantly different among themselves. The seed stored for up to 678 days at -5 and 5°C and reduced moisture content took significantly low MGT of 9.05 and 9.49 days respectively as compared to 9.71 and 11.48 days with original moisture content.

The effect of temperature and moisture content on early seed deterioration with progress of the storage period was not detected always by germination percentage alone particularly under conditions where seed showed fairly good storability, until significant reduction in seed vigour was detected. In the present investigation, the GI and EC quantified the vigour of seed stored for upto 678 days. The decline in GI was steep when seed at 10.46% moisture content was stored at RT (figure 2). Seed stored at -5

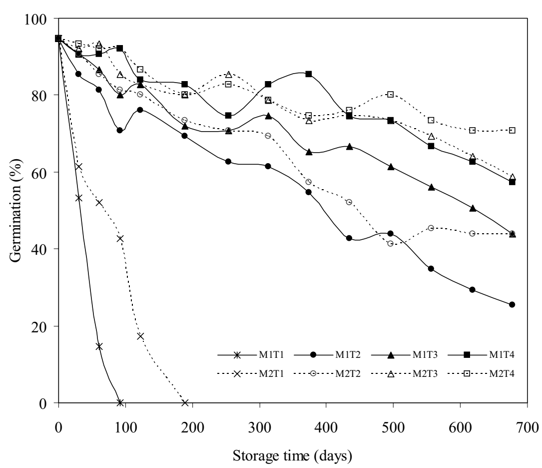

Figure 1. Germination of *U. wallichiana* seed in relation to seed moisture content (M1=10.46%, M2=3.09%), storage temperature (T1=RT, T2=15°C, T3=5°C, T4=-5°C) and days in storage.

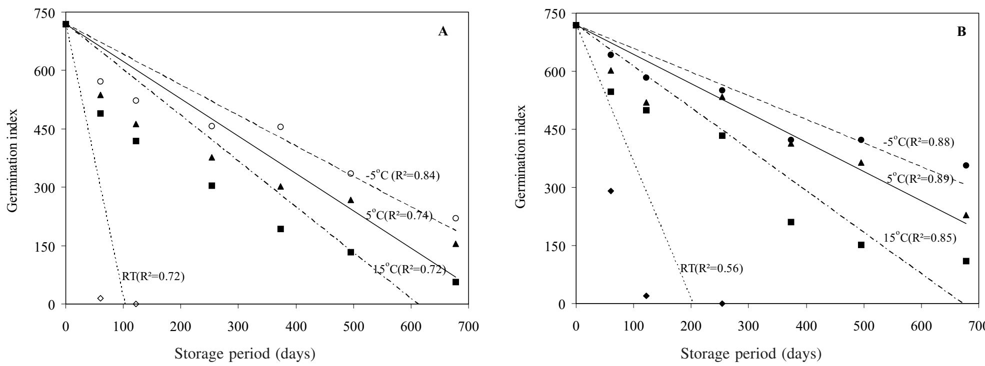

Figure 2. The declining pattern of seed vigour (germination index) with storage time for *U. wallichiana* seed stored at different temperatures with (A) original (10.46%) and (B) reduced (3.06%) moisture content in sealed container.

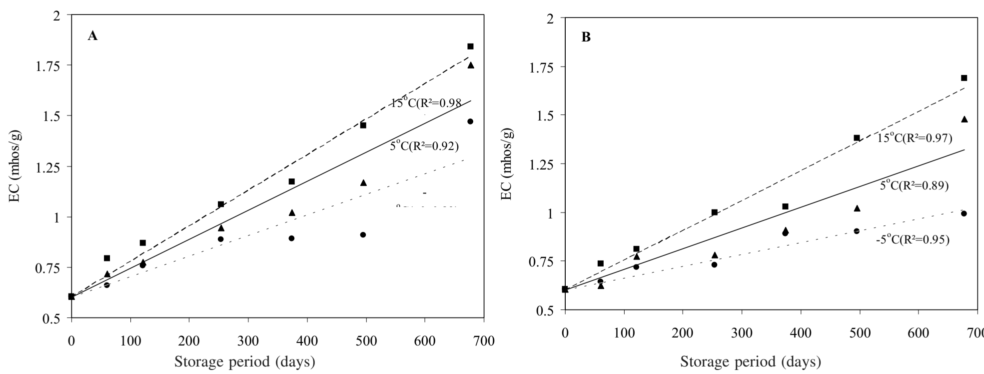

Figure 3. The declining pattern of seed vigour (EC) with storage time for *U. wallichiana* seed stored at different temperatures with (A) original (10.46%) and (B) reduced (3.06%) moisture content in sealed container.

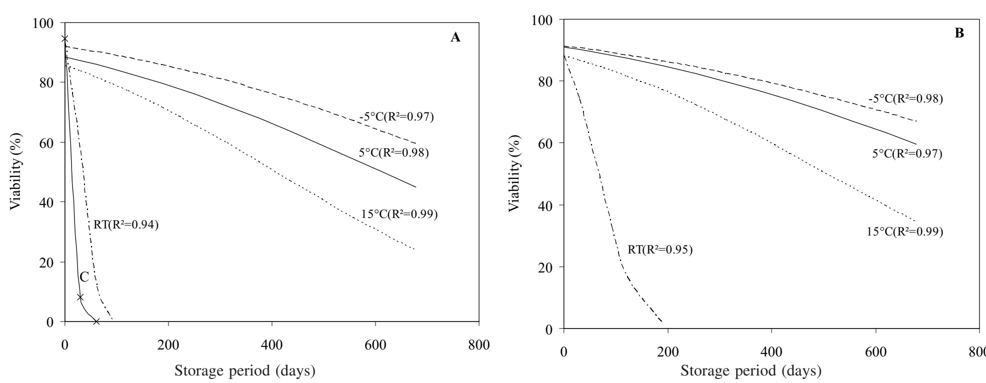

Figure 4. Survival curve for *U. wallichiana* seed stored at different temperatures with (A) original (10.46%) and (B) reduced (3.09%) moisture content in sealed container. C = seed stored in open container at RT with original moisture content (control).

and 5°C with 3.09% moisture content showed lesser decline in GI as compared to seed stored with 10.46% moisture content at the same temperature. Electrical conductivity of seed leachates increased progressively as storage temperature and seed moisture content increased (figure 3).

The survival curves for seed stored at 15, 5 and -5°C with 3.09% moisture content showed lesser gradient as compared to the seed stored with 10.46% moisture content at similar storage temperatures (figure 4). The slope of survival curve reflects that decrease in seed moisture content as well as storage temperature proportionally decreased the rate of deterioration (table 1). It was substantially high (1.1171) at RT with 10.46% moisture content followed by 0.5131 at the same temperature with 3.09% moisture content. The minimum rate of deterioration of 0.0329 was recorded at -5°C with 3.09% moisture content. An overall view of p_{50} in various treatments showed that it increased linearly with reduced seed moisture content and storage temperature (table 2). The maximum p_{50} of 998 and 828 days was observed at -5 and 5°C with low seed moisture content of 3.09%. Seed stored as control at RT with original moisture content in open container showed minimum p_{50} of 16 days.

Table 1. The effect of seed moisture content and storage temperature on the rate of deterioration in *U. wallichiana* seed.

Storage temperature	Rate of deterioration (d^{-1})	
	Seed moisture content	
	10.46%	3.09%
RT	1.1171	0.5131
15°C	0.0892	0.0750
5°C	0.0598	0.0404
-5°C	0.0432	0.0329

Rate of loss of viability was 1.8124, when seed was stored in open container at ambient room temperature with original moisture content (control).

Table 2. The half viability period (p_{50}) of *U. wallichiana* seed at different moisture content and storage temperatures.

Temperature	Moisture content (%)	p_{50} (days)
RT	10.46	034
15°C	10.46	406
5°C	10.46	612
-5°C	10.46	817
RT	03.09	067
15°C	03.09	506
5°C	03.09	828
-5°C	03.09	998

p_{50} of 16 days was observed in seeds stored in open containers at ambient room temperature with original seed moisture content (control).

Discussion

Ulmus wallichiana seeds lose viability within a few months under natural conditions or if stored in open containers at ambient room temperature. Though the seed appears to be short lived in the first instance, results reveal that they could be stored for several years at sub-zero (-5°C) temperature in dry conditions similar to the seed of *Ulmus americana* (Barton, 1961), *Populus balsamifera* and *Salix galuca* (Zasada and Densmore, 1980). Seeds of *U. wallichiana* exhibit a well-defined pattern of an increase in longevity with decrease in storage temperature and seed moisture content. This is indicated from the shape of survival curves and rate of deterioration (d^{-1}) quantified from the regression coefficient for slope to determine vigour of *U. wallichiana* seed. Seed with high moisture content remained viable for comparatively shorter periods than seed with low moisture content. This trend was appeared at all the temperatures. The importance of moisture content alone in maintaining viability is evident in seed stored at RT. The reduction in moisture content from 10.46 to 3.09% increases the value of p_{50} from 34 to 67 days at RT. Thus, the seed of *U. wallichiana* prove to be desiccation tolerant, at least to a moisture content of about 3.09% and can be stored at low temperatures. This desiccation tolerance is a close agreement with the *U. carpinifolia* (Tompsett, 1986) and *U. americana* (Barton, 1939, 1953) seeds which tolerate desiccation to 3 and 2% respectively. Similar results have been reported in seed of *Ulmus laevis* (Tylkowski, 1987), *U. pumila* (Heit, 1967), *Acer caesium* (Phartyal *et al,* 2003), *Dendrocalmus brandisii* (Boonarutee and Somboon, 1990), *Bambusa arundinacea* (Somen and Seethalakshmi, 1989) and *B. tulda* (Thapliyal *et al.,* 1991). The longevity of *Araucaria columnaris* seeds was increased by reducing seed moisture content from 22 to 7% and storage temperature from 36 to 21°C (Tompsett, 1984). Poulsen (1993) reported that drying to 7% moisture content and temperature below 0°C increased the storability of European beechnut (*Fagus sylvatica*) seed for 3-4 years.

Usually, ageing in seeds has been quantified by the progressive loss of germination percentage. In *U. wallichiana* loss in vigour appeared a more sensitive assay of seed deterioration in storage as compared to seed viability (germination), which for different samples of stored seed drawn on different dates remain more or less equal and was at par statistically. Loss in vigour can be thought of as an intermediate stage in the life of the seed, occurring between the onset and termination of viability and germination. The MGT, EC and GI further quantify the seed vigour of *U. wallichiana* during storage. All above parameters reveal that seed vigour decreases concomitantly with storage period, as well as with seed moisture content and storage temperature increases. The change in GI is used to indicate the optimum storage condition where loss of seed vigour is least (figure 2). The EC increases with the progress of deterioration during storage in different conditions (figure 3). This is related to varying vigour and consequent loss of viability in seed due to disintegration of cell membranes and low ability to repair. Bonner (1991) recommended EC as a rapid and non-destructive test for the objective estimates of seed quality of most southern pines. An increase in EC with the loss in viability has been shown in seeds of several other species (Marcus-Filho and Mcdonald, 1998). In accelerated aged seed of *Dalbergia sissoo,* decrease in viability and vigour was accompanied by progressive increase in EC (Thapliyal and Connor, 1997) and MGT (Joshi, 2000).

From the overall storage behaviour of *U. wallichiana* seed, it may be concluded that this species fits in the definition of "orthodox" seed proposed by Roberts (1973), with respect to the relation between seed moisture content, storage temperature and seed longevity as it survives desiccation to a low moisture content of 3.09% and low storage temperature of –5°C.

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