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# Effect of seed moisture content and storage temperature on seed longevity of hemp (*Cannabis sativa*)

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ABSTRACT

Hemp (*Cannabis sativa* L.) is one of the earliest domesticated plants grown for its protein and oil rich seed, fiber and psychoactive substances and it is one of the earliest known medicinal plants in human history. Studies were conducted on seed germinability (germination test) and viability (topographical tetrazoliun chloride test) in three seed lots to determine the seed quality. Studies conducted on effect of five seed moisture contents (5, 7, 8, 10 and 12 % on fresh weight basis), three storage temperature (ambient, 15°C and -20°C) and eight storage periods (0, 3, 6, 9, 12, 18, 24 and 36 months) on seed longevity revealed that the critical moisture content (moisture content required in seeds for retaining initial germination after storage of seeds up to 36 months) of seeds for ambient storage condition of Delhi was 5 %, which increased to 7 % in 15°C and 12 % at -20°C storage temperature. The seeds are desiccation as well as chilling tolerant, therefore, exhibit orthodox storage behavior and are ideal for *ex-situ* conservation of seeds in seed/gene banks.

**Key words:** *Cannabis sativa*, Hemp, Orthodox seeds, Storage behaviour

About 7.5 million samples of seeds are stored in 1750 National, International and private seed banks since 1970s, because storage of seeds in seed banks/gene banks is generally considered the safest, most inexpensive and most convenient method of conservation as seeds occupy little space, and also they require little attention over considerable period of time. Conservation of germplasm only in field condition is risky as it can be lost because of genetic erosion, pest or disease or adverse weather conditions (Engelmann and Engels 2002). However, storage of seeds in seed banks for *ex-situ* conservation needs a thorough understanding of post harvest seed physiology as seeds exhibiting orthodox seed storage behaviour (desiccation tolerant) can only be stored in seed banks for a longer period of time without losing the seed viability. A sizeable section of economically important plants produce seeds that are termed as recalcitrant or desiccation sensitive seeds and can not be stored in seed/gene banks like orthodox seeds. The ability of many orthodox seeds (Roberts 1973) to remain viable for a long period of time make them convenient for the long-term *ex situ* conservation of plant germplasm in seed/gene banks as the longevity of seeds in storage is mainly determined by seed moisture content and storage temperature, with life-span increasing predictably with decreasing temperature and moisture content (Harrington 1972, Ellis and Roberts 1980). However, there are also wide inherent differences in seed longevity between species (Harrington 1972, Priestley *et al.* 1985). For example, the predicted time for viability to decline from 97.7% to 84.1% for seeds stored under gene-bank conditions ranges from approx. 30 years for *Ulmus carpinifolia* to approx. 6000 years for *Sorghum bicolor* (Probert *et al*. 2009). However, there is already evidence that some species produce seeds with much shorter longevity in dry storage. For example, seeds (with high initial viability) of *Anemone nemorosa* are predicted to survive, 1 year under seed bank storage conditions (Ali *et al.* 2007). Understanding species differences in seed longevity is therefore crucial to the effective management of seed conservation collections because it underpins the selection of viability re-test intervals and hence regeneration or re-collection strategies.

*Cannabis sativa* L. (marijuana, hemp; *Cannabaceae*), one of the most widely distributed cultivated plant and one of the earliest domesticated plant species, has been used for millennia as a source of fibre, oil- and protein-rich seeds and for its medicinal and psychoactive properties (Courtwright 2001) and it is one of the earliest known medicinal plant in human history (Zias *et al*. 1993). *C. sativa* is cultivated in higher altitude of Uttarakhand State of India (Uttarakhand extends from 28º 43' N to 31º 27' N longitude and 77º 34' East to 81º 02' E latitude) as a rainy

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season crop (July to November) for its seeds and fibre and plays an important role in the economy of rural people, where other crops can not be grown with such little inputs. Although *C. sativa* is suspected to exhibit orthodox storage behaviour (Liu *et al*. 2008), but detailed information on seed longevity in relation to seed moisture content and storage temperature is not known. Therefore, studies were under-taken with the aim to determine the (i) per cent germination in different incubation temperatures and time taken for germination in three seed lots procured from open market of Bageshwar, Uttarakhand, (ii) per cent viable seeds in the three seed lots using topographical tetrazolium chloride test and (iii) effect of different seed moisture content (mc) and storage temperature on seed longevity.

## MATERIALS AND METHODS

Three seed lots of one kg each were purchased from local market of Bageshwar, Uttarakhand ( lat 29.83, long 79.773 and alt 1500 m). The hemp seeds are made available to the local market by the farmers, which are grown in the higher altitude (1500 to 2500 m MSL) of the region. Seeds are used as spice, food additives and various other kind of culinary preparations by the local inhabitants. Three seed lots purchased from the market were cleaned using a seed blower and processed using a gravity separator. Under sized and over sized seeds comprising about 8 to 15 % and 3 to 7 % (by weight) in different seed lots, respectively were discarded so as to obtain a homogenous seed lot for subsequent studies.

Germination tests were conducted using between paper (BP) method (rolled towel test) in 400 seeds (100 seeds in four replications) in five incubation temperatures, viz. 15, 20, 25, 20-30 (higher temperature for six hours), 30 and 35°C so as to identify the optimum temperature for germination and time taken for germination. Seeds were considered germinated when all the essential structures of seedlings were visible in order to differentiate between normal seedlings from abnormal seedlings and percent germination was determined based on percent normal seedlings. Abnormal seedlings were categorized on the basis of root and shoot structure. Seedlings with abnormal roots (primary root stunted, stubby, missing, glassy, with negative geotropism, decayed due to primary infection) or abnormal shoots ( shoot short and thick, missing, constricted, twisted or decayed due to primary infection, cotyledonary leaves deformed, damaged or decayed as a result of primary infection) were considered as abnormal seedlings. Percent germination was expressed in nearest whole number as per standard practice used in seed trade (ISTA 2008). Seedlings were removed on first count when more than 50 % seeds germinated and final count was taken for the remaining seedlings which germinated after seedlings were removed on first count and time required for completing germination was recorded for each replication/treatment.

Viability test was conducted to distinguish between non-viable (dead) and dormant seeds (as the germination was low in seed lot 2 and 3 and the seeds were non-viable but not dormant as a viable seed may not germinate due to dormancy in a germination test) following ISTA (2003). 400 seeds were soaked in water at 20°C for 18 hrs. The pericarp was opened by gently pressing the seeds from the lateral side and the embryo was removed. The thin and papery seed coat was also removed with the help of forceps. The excised embryos were soaked in 1% TZ solution (2,3,5 triphenyl tetrazolium chloride) at 30°C for 18 hrs. The red coloured stained embryos were observed in a stereoscopic microscope for staining pattern of the embryonic axis and cotyledons. The embryos were separated into (i) viable (totally stained) or more than 1/3 of radicle or cotyledons stained and (ii) non-viable, i.e. totally unstained or partially stained = 1/3 of the radicle (measured from the tip) or 1/3 of cotyledon unstained.

Moisture content in seeds was determined using 5 g seeds in each lot (two replications) by drying the seeds at 103°C for 17 hrs (ISTA 2008). 1000 seed weight was determined by weighing 100 seeds in 8 replications (ISTA 2008) and oil content was determined using Soxhlet method (AOAC 2005).

The processed and homogenous seed lot no. 1 having 8.22 % moisture content with > 90 % germination and 98 % seed viability (Table 1) was again dehydrated / moistened using lithium chloride solutions (Gold and Hay 2008) in

Table 1 Effect of different temperatures on germination, seed viability moisture and oil content and 1000 seed weight (TSW)

| Seed lots | Incubation temperatures (°C) | | | | Mean | Seed viability (%) | Moisture content (%) | Oil content (%) | TSW (g) |
|---|---|---|---|---|---|---|---|---|---|
| | 20 | 25 | 20–30 | 30 | | | | | |
| 1 | 94 | 92 | 91 | 13 | 72.5 | 98 | 8.22 | 34.32 | 23.82 |
| | (75.8)* | –73.5 | –72.5 | –21.1 | –60.7 | –81.8 | | | (2.15)** |
| 2 | 76 | 78 | 74 | 15 | 60.7 | 82 | 9.56 | 35.57 | 18.68 |
| | –60.6 | –62 | –59.3 | –22.7 | –51.1 | –64.9 | | | (3.23)** |
| 3 | 64 | 61 | 83 | 0 | 52 | 88 | 8.76 | 36.53 | 21.56 |
| | –53.13 | –51.3 | –65.6 | | –42.5 | –69.7 | | | (2.87)** |
| Mean | 78 | 77 | 82.6 | 9.3 | – | 89.3 | 8.84 | 35.47 | 21.35 |
| | –63.1 | –62.2 | –65.8 | –14.6 | | –72.1 | | | |
| CD | Lots 2.56, temperature 3.56 | | | | | | 6.54 | | |
| (P = 0.05) | L × T 4.65 | | | | | | | | |

*Figure in parenthèses are arc sine % transformation, ** coefficient of variation

Table 2 Effect of seed moisture content and storage period on germination (%) of seeds stored under ambient storage condition

| Storage period (months) | Moisture content (mc) in seeds (%) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 5 | 7 | 8 | 10 | 12 | |
| 0 | 88 (69.73) | 86 (68.03) | 90 (71.56) | 85 (67.21) | 87 (68.87) | 87.2 (69.04) |
| 3 | 86 (68.03) | 80 (63.44) | 40 (39.23) | 5 (12.92) | 0 | 42.2 (40.51) |
| 6 | 82 (64.90) | 45 (42.13) | 0 | 0 | 0 | 25.4 (30.26) |
| 9 | 83 (65.65) | 0 | 0 | 0 | 0 | 16.6 (51.12) |
| 12 | 85 (67.21) | 0 | 0 | 0 | 0 | 17.0 (24.35) |
| 18 | 83 (65.65) | 0 | 0 | 0 | 0 | 16.6 (24.04) |
| 24 | 86 (68.03) | 0 | 0 | 0 | 0 | 17.2 (56.91) |
| 36 | 84 (66.42) | 0 | 0 | 0 | 0 | 16.8 (51.24) |
| Mean | 84.6 (66.89) | 26.3 (30.85) | 16.2 (23.73) | 11.2 (19.55) | 10.8 (19.19) | |

CD (P = 0.05) Moisture content 3.54 storage period 2.24 , mc × storage period 4.32

desiccators and seeds with targeted moisture content (mc) of 5, 7, 8, 10 and 12 % were prepared using the following equation (Danida Forest Centre 1999):

$W_2 = (100\text{-}A) / (100 - B) \times W_1$ ; where $W_2$ is the mass of seed (g) of targeted moisture content, A is the initial moisture content, B is the targeted moisture content and $W_1$ is the initial seed mass (g). The water loss/gain in seeds were monitored by weighing the seeds at regular intervals.

Seeds with five targeted mc were stored in hermetically sealed containers in three storage temperatures, viz. (a) ambient laboratory condition-where temperature varied from 10°C in winter to 40°C in summer (b) at 15°C and (c) at -20°C in a refrigerator. Seed longevity (in seeds with different mc) was determined by testing seeds using BP method at 25°C before storage (after preparing the seeds with desired targeted moisture content) and after storage at 0, 3, 6, 9, 12, 18, 24 and 36 months interval following Hong & Ellis (1996) (the experimental design consisted of six moisture contents, three storage temperatures and eight storage periods). Seed vigour as reflected by seedling length was determined on the first count (4th day) using 20 seedlings per replication selected randomly. Light was provided for 6 hr in all the germinating temperatures. Seeds with 5 and 7 % mc were humidified for 24 hr in 90 % RH in desiccators (maintained by using lithium chloride solution) to avoid imbibitional injury due to rapid uptake of water (Powell and Mathew 1978). Statistical analysis was carried out using WASP – Web Agri Stat Package (http://icargoa.res.in/wasp/index.php).

## RESULTS AND DISCUSSION

Germination begins with the uptake of water by the quiescent seed as embryonic axis elongates leading to the protrusion of radicle through pericarp within 24 hr of sowing and all the essential structure of seedlings become differentiated within 3-4 days (when >80 to 90 % seeds germinate) and no germination take place after 7 days of seed sowing. Seeds of hemp are non-endospermic and non-dormant with a high embryo seed (E:S ratio > 0.9) ratio (i.e. the relative size of the embryo within the seed) as embryo fills up almost all the seed volume with no nutritive storage tissue. Germination is epigeal and it is one of the fastest germinating crop (having a test duration of 6-7 days) compared to other crops, where test duration is much longer (ISTA 2008). Seeds with low E:S ratio takes longer time for germination and usually exhibit morphological or physiological dormancy (Baskin and Baskin 2004, Linkies *et al.* 2010). There seems to be a general trend of increasing relative embryo size during evolution (higher E : S ratio) in angiosperms and evolution of larger embryo size possibly resulted in occurrence of non-dormant seeds (Finch-Savage and Leubner-Metzer 2006). The E:S ratio have increased in derived angiosperms compared to ancestral angiosperms (Forbis *et al*. 2002).

Table 3 Effect of seed moisture content and storage period on germination (%) of seeds stored at 15°C.

| Storage period (months) | Moisture content (mc) in seeds (%) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 5 | 7 | 8 | 10 | 12 | |
| 0 | 88 (69.73) | 92 (73.57) | 88 (69.73) | 88 (69.73) | 88 (69.73) | 88.8 (70.45) |
| 3 | 86 (68.03) | 85 (67.21) | 86 (68.03) | 85 (67.21) | 80 (63.44) | 84.4 (70.09) |
| 6 | 82 (64.90) | 88 (69.73) | 87 (68.87) | 72 (58.05) | 62 (51.94) | 78.2 (69.91) |
| 9 | 86 (68.03) | 90 971.56) | 88 (69.73) | 69 (56.15) | 61 (51.35) | 78.8 (62.58) |
| 12 | 90 (71.56) | 87 (68.87) | 85 (67.21) | 54 (47.29) | 43 (40.98) | 71.8 (57.92) |
| 18 | 84 (66.42) | 80 (63.44) | 76 (60.67) | 48 (43.85) | 23 (28.66) | 62.2 (52.06) |
| 24 | 86 968.03) | 85 (67.21) | 61 (51.35) | 41 (39.82) | 0 | 54.6 (47.64) |
| 36 | 84 (66.42) | 88 (69.72) | 63 (52.53) | 43 (40.98) | 0 | 55.6 (48.22) |
| Mean | 85.7 (67.78) | 86.8 (68.70) | 79.25 (62.9) | 62.5 (52.24) | 44.6 (41.9) | - |

CD (P = 0.05) mc 2.32, storage period 2.43, mc × storage period 3.54

Table 4 Effect of seed moisture content and storage period on germination (%) of seeds stored at –20 °C

| Storage period (months) | Moisture content (mc) in seeds (%) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 5 | 7 | 8 | 10 | 12 | |
| 0 | 92 (73.57) | 90 (71.56) | 87 (68.87) | 88 (69.73) | 90 (71.56) | 89.4 (71.88) |
| 3 | 87 (68.87) | 91 (72.54) | 88 (69.73) | 87 (68.87) | 89 (70.63) | 88.4 (70.09) |
| 6 | 90 (71.56) | 88 (69.73) | 90 (71.56) | 84 (66.42) | 86 (68.03) | 87.6 (69.38) |
| 9 | 88 (69.73) | 90 (71.56) | 87 (68.87) | 89 (70.63) | 89 (70.63) | 88.6 (70.27) |
| 12 | 90 (71.56) | 88 (69.73) | 88 (69.73) | 87 (68087) | 85 (67.21) | 87.6 (69.38) |
| 18 | 87 (68.87) | 85 (67.21) | 89 (70.63) | 85 (67.21) | 84 (66.42) | 86.0 (68.03) |
| 24 | 91 (72.54) | 87 (68.87) | 88 (69.73) | 86 (68.03) | 88 (69.76) | 88.0 (69.76) |
| 36 | 90 (71.56) | 90 (71.56) | 85 (67.21) | 86 (68.03) | 85 (67.21) | 87.2 (69.04) |
| Mean | 89.37 (71.1) | 88.62 (70.2) | 87.75 (69.3) | 86.5 (68.44) | 87 (68.87) | - |

CD (P = 0.05) mc NS, storage period NS

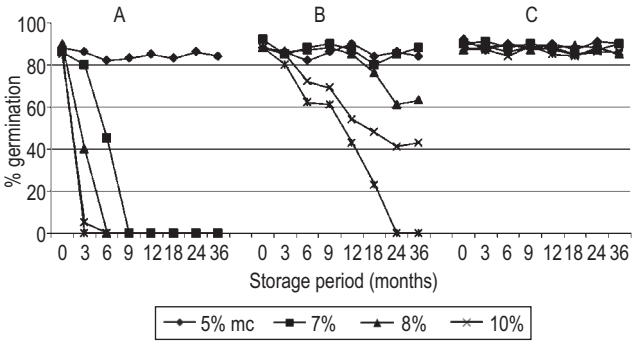


Fig 1 Effect of seed moisture content and storage period on % germination under (A) ambient storage (B) at 15°C and (C) at –20 °C.

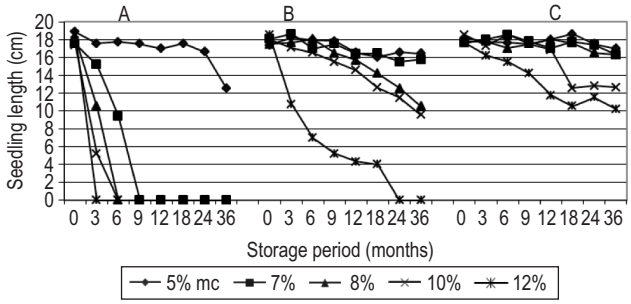


Fig 2 Effect of seed moisture content and storage period on seedling lenth (A) seeds stored under ambient condition (B) at 15°C (C) at –20°C

Perusal of data in Table 1 reveals that no significant difference in final germination was observed in 20, 25 and 20-30°C. No germination was observed in 15°C and thermo-inhibition of germination was recorded in 30 and 35°C . First count was conducted on 4th day (when all essential structure of seedlings were visible in > 80 % seeds) and final count on 7th day (no germination was recorded after 7th day onwards in any seed lot at any temperature). Seed viability as well as seed germinability was highest in seed lot 1, i.e 98 and 94 % respectively. Freshly harvested seeds were non-dormant as confirmed by TZ test as percent viable and germinable seeds were almost equal. Moisture content (on fresh weight basis) as well as oil content varies in seed samples (Table 1). 1000 seed weight also varied (Table 1) and co-efficient of variation was less than 4 in all the three seed lots, which is within the range for non-chaffy seeds (ISTA 2008).

*Effect of seed moisture content, storage temperatures and storage periods on seed viability and vigour:* Seed viability is influenced by diverse factors such as plant species, environmental conditions during seed development and maturation, physiological status of the seed at maturity and seed storage methods (Probert and Hay 2000, Pritchard and Dickie 2004 and Walters *et al*. 2005). However, longevity of stored seeds is mainly affected by seed moisture content, temperature and oxygen concentration in the storage environment (Roberts 1972). Perusal of data in Fig. 1 and 2 and Table 2 to 7 reveals that seed longevity as well as seed vigour (as reflected by seedling length) declined rapidly under ambient storage condition in seeds with higher moisture content and seed viability and vigour increased with the decrease in the storage temperature and moisture content. Under ambient storage condition, seeds with 5 % mc could retain the initial viability up to 36 months of storage, while seeds with higher mc lost viability within nine months of storage (Fig 1 A and Table 2) suggesting

Table 5 Effect of seed moisture content (%) and storage period on seedling length (cm) of *C. sativa* seeds stored under ambient storage condition

| Storage period (months) | Moisture content in seeds (%) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 5% mc | 7% | 8% | 10% | 12% | |
| 0 | 18.88 | 17.52 | 18.26 | 17.41 | 18.67 | 18.14 |
| 3 | 17.56 | 15.22 | 10.53 | 5.23 | 0 | 9.7 |
| 6 | 17.76 | 9.42 | 0 | 0 | 0 | 5.43 |
| 9 | 17.54 | 0 | 0 | 0 | 0 | 3.5 |
| 12 | 16.98 | 0 | 0 | 0 | 0 | 3.39 |
| 18 | 17.56 | 0 | 0 | 0 | 0 | 3.51 |
| 24 | 16.64 | 0 | 0 | 0 | 0 | 3.32 |
| 36 | 12.56 | 0 | 0 | 0 | 0 | 2.51 |
| Mean | 16.93 | 5.27 | 3.59 | 2.83 | 2.33 | |

CD (P=0.05) mc 1.54, storage period 0.53, mc × storage period 3.21

Table 6 Effect of seed moisture content (%) and storage period on seedling length (cm) of *C. sativa* seeds stored at 15°C

| Storage period (months) | Moisture content in seeds (%) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 5% | 7% | 8% | 10% | 12% | |
| 0 | 17.41 | 18.05 | 17.51 | 18.05 | 18.54 | 17.91 |
| 3 | 18.23 | 18.65 | 17.65 | 17.08 | 10.78 | 16.47 |
| 6 | 17.98 | 16.98 | 18.06 | 16.56 | 6.95 | 15.3 |
| 9 | 17.85 | 17.53 | 16.5 | 15.52 | 5.22 | 14.52 |
| 12 | 16.53 | 16.43 | 15.7 | 14.56 | 4.33 | 13.51 |
| 18 | 16.05 | 16.45 | 14.2 | 12.6 | 4.01 | 12.66 |
| 24 | 16.58 | 15.5 | 12.56 | 11.5 | 0 | 11.22 |
| 36 | 16.45 | 15.8 | 10.54 | 9.56 | 0 | 10.47 |
| Mean | 17.13 | 16.92 | 15.34 | 14.42 | 6.22 | |

CD (P=0.05) mc 1.66, storage period 1.63 mc × storage period 2.23

Table 7 Effect of seed moisture content (%) and storage period on seedling length (cm) of C. sativa seeds stored at -20°C.

| Storage period (months) | Moisture content in seeds (%) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 5% | 7% | 8% | 10% | 12% | |
| 0 | 18.21 | 17.62 | 17.77 | 18.55 | 17.67 | 17.96 |
| 3 | 17.78 | 18.04 | 17.82 | 17.32 | 16.23 | 17.43 |
| 6 | 17.67 | 18.55 | 17.05 | 18.55 | 15.54 | 17.47 |
| 9 | 17.55 | 17.78 | 17.55 | 17.52 | 14.23 | 16.92 |
| 12 | 18.01 | 17.05 | 18.02 | 16.88 | 11.78 | 16.34 |
| 18 | 18.67 | 17.76 | 17.63 | 12.56 | 10.56 | 15.43 |
| 24 | 17.55 | 17.41 | 16.54 | 12.83 | 11.54 | 15.17 |
| 36 | 17.01 | 16.34 | 16.32 | 12.65 | 10.25 | 14.51 |
| Mean | 17.80 | 17.56 | 17.33 | 15.85 | 13.47 | |

CD (P=0.05) mc 1.21, storage period 1.56, mc x storage period 3.21

that higher ambient temperature of Delhi is too high for safe seed storage of hemp seeds. Seed vigour as reflected by seedling length was also not affected up to 24 months (Fig. 2 A and Table 5), although significant reduction in seed vigour was recorded after 24 months, suggesting that seeds with 5 % mc can be safely stored up to 36 months in hermetically sealed containers under ambient condition of Delhi (IARI, New Delhi-Latitude: 28°38' 23"N Longitude: 77°09'27"E, Altitude: 228.6m above msl). The moisture content of the seeds was reduced using Li $Cl_3$ (Gold and Hay 2008) as the moisture content of seed when procured varied from 8.22 to 9.56 % suggesting that reduction of seed mc below 8 % is not possible under ordinary sun drying process as practiced by farmers of the hemp growing region in Himalayas (as seed mc will equilibrate with prevailing RH). However, seeds with 8 to 9 % mc retain viability for next seed sowing season for about 6-7 months because of low temperature prevailing in the hemp growing region in Uttarakhand (which rarely exceeds 20 to 22 °C during summer) as the seeds are harvested during October-November and stored up to June –July for next sowing at the onset of monsoon.

Storage of seeds at 15°C (Fig 1B and 2B and Table 3 and 6) revealed no significant difference in percent germination in seeds with 5 and 7 % mc up to 36 months, while seeds with 8 % mc retained initial germination up to 12 months storage. Complete loss of viability was observed in seeds with 12 % mc after 24 months, while seeds with 10 % mc retained > 40 % germination after 36 months of storage at 15°C. Seed vigour (Fig 2B and Table 6) was also maintained throughout storage period of 36 months in seeds with 5 and 7 % mc, while it was reduced in seeds with higher mc. Storage of seeds at –20°C (Fig 1C and 2C and Table 4 and 7 ) revealed that seeds are desiccation as well as chilling tolerant as no significant difference in germination was observed in seeds with different mc although reduction in seed vigour was evident in seeds with 10 and 12 % mc (Table 7).

Generally orthodox seed longevity is assumed to increase as seed moisture content is lowered and storage temperatures decrease (Ellis and Roberts 1980, Ellis 1998), although lettuce (*Lactuca sativa*) seeds can maintain viability with a seed mc up to 15% (Ibrahim and Roberts 1983). Further research has shown a relationship between seed composition (oily versus non-oily seed), percent moisture content (mc) and longevity during storage (Roberts and Ellis 1989, Lehner *et al*. 2006). Roberts and Ellis (1989) found that during low temperature storage, viability could be maintained in oily seeds (lettuce) as long as mc was less than 15%, whereas in non-oily seed like barley (*Hordeum vulgare*)) viability could be maintained with seed mc as high as 28%. Research examining the thermodynamic properties of water in pea (*Pisum sativum*) seeds (Vertucci and Roos 1991, Vertucci *et al.* 1994), and molecular mobility of water in impatiens (*Impatiens walleriana*), pea and cattail (*Typha latifolia*) seeds (Buitink *et al.* 2000) suggests that there is an optimum mc for each storage temperature, which increases as storage temperature decreases. This mc is proposed to correspond to one that minimizes cellular fluidity, but with water present in sufficient quantities for maintaining protective effects (Vertucci and Roos 1991, Buitink *et al.* 2000).

Although reduction in moisture content and storage temperature increases the life span of seed, but it can not be prolonged indefinitely by progressively drying the seeds (Ellis 1998, Probert and Hay 2000) and moisture content below which longevity could not be improved is considered the critical moisture content (Ellis and Hong 2007). The existence of the critical moisture content is the most important point in the seed storage debate and drying below certain moisture content will not improve seed longevity (Buitink & Hoekstra 2004). The critical moisture content for storage depends on storage temperature and chemical composition of seeds. The critical moisture content of hemp seeds under ambient storage condition (up to 36 months) appears to be 5 % as seeds with >5 % mc lost germinability within nine months of storage (Fig 1A and Table 2), although significant reduction in seed vigour (as reflected by seedling

length) was observed after 24 months of storage (Fig 2A and Table 5). The critical mc increased to 7 % at 15°C storage temperature (Fig 1B and 2B) and to 12 % at – 20°C ( Fig 1C and 2C). However, reduction in seed vigour at –20°C in seeds having 10 and 12% mc may be attributed to ageing of seeds due to higher moisture content in hemp seeds. No reduction in seed vigour was observed in seeds with 5 and 7 % mc in 15°C storage temperature (Fig 2B) suggesting that seeds of hemp can be stored safely in seed banks for at least up to 36 months, without any loss to vigour as well as longevity. Seeds of hemp could not survive with >5 % mc under Delhi condition (where the prevailing temperature is much higher during March to June than the places where the crop is grown in higher altitude of Uttarakhand, India), as the crop is harvested during November to December and again sown during June to July and the viability of the seeds (with 8-9 % mc) is retained for at least one season (six months) under the ambient storage condition of higher altitude of Uttarakhand region. Hemp seeds are rich in oil (c. 35 %) and oxidation of unsaturated fatty acids is considered to be primary reaction in aging of seeds, contributing to free radicle production and subsequent attack to macromolecules (Pritchard and Dikie 2004, Rajjou and Debeaujon 2008).

Increased longevity upon drying is related to an increased intracellular viscosity and dehydration induced increase in cytoplasm viscosity leads to a decrease in the rate of detrimental reaction in seeds leading to an increase in seed longevity. When seed mc falls below a certain value, the cytoplasm becomes so viscous that it transforms into a so-called glass. A glass is a thermodynamically unstable solid state with an extremely high viscosity and low moisture in seed and low storage temperature promotes its formation (Buitink and Hoekstra 2004). The processes that take place in dry seed and lead to after-ripening or seed deterioration are an important aspect of seed biology and the topic of active research as even small changes in overall seed moisture content influence storability and longevity of seeds (Nagel and Borner 2010). Understanding these processes is an issue of economic importance and a major concern for seed quality assurance and also for ex-situ conservation of seeds in seed banks.

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