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# Seed storage behaviour in *Berberis aristata*

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ABSTRACT

*Berberis aristata* DC (of *Berberidaceae*) is commonly known as Daruharidra or Daruhaldi. It is one of the most valuable medicinal plants in Indian system of medicine. No information is available on seed morphology, dormancy and seed storage behavior of the species which is required for conserving the seeds in seed banks. Therefore an experiment was conducted with seed lot collected from Almora, Uttarakhand. Studies were conducted on seed germinability (using germination tests) and seed viability (using topographical tetrazolium chloride test) in relation to desiccation and chilling tolerance using a factorial combination of four moisture content (5, 7, 10 and 12% moisture content in seeds on fresh weight basis), three storage temperatures (seeds stored in ambient condition, at 15°C and at –20°C) and five storage periods (0, 3, 6, 9 and 12 months). Perusal of data revealed that no significant loss in germination was observed in seeds with 5 and 7% moisture content under ambient as well as other storage conditions, suggesting that seeds of *Berberis aristata* are desiccation as well as chilling tolerant and exhibited orthodox seed storage behavior. The seeds are ideal for *ex situ* conservation in seed banks/ gene banks for a longer period.

**Key words:** *Berberis aristata*, Intermediate seeds, Orthodox seeds, Recalcitrant seeds, Seed storage

Millions of seed samples are stored in 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 (Linington and Pritchard 2001, Engelmann and Engels 2002). 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. However, storage of seeds in seed banks for *ex-situ* conservation needs a thorough understanding of post harvest seed physiology and seed storage biophysics as seeds exhibiting orthodox seed storage behaviour can only be stored in seed banks for a longer period of time without loosing the seed viability. Orthodox seeds are desiccation tolerant and can be dried to 5-7 % moisture content (fresh weight basis) and stored at sub-zero temperature. Seed survival in desiccation tolerant seeds can be quantified by seed viability equations, as seed viability depends upon chemical composition of seed, moisture content and storage temperature (Roberts 1973, Ellis and Roberts 1980, Ellis *et al*. 1988, Pritchard and Dickie 2004, Hong *et al.* 2005). Orthodox seeds retain viability for several decades in gene bank condition as 90-95 % of their original water is lost during seed maturation. However, a sizeable section of economically important plants especially trees and shrubs produce seeds that differ in their capacity to withstand water loss and termed as recalcitrant or desiccation sensitive seeds. Recalcitrant (desiccation sensitive) seeds do not undergo maturation drying and cannot withstand water loss of the magnitude of that experienced by orthodox seeds. In order to remain viable, they must not undergo any substantial change in moisture (Berjak and Pammenter 1997, 2003). Placing seeds with high moisture content at sub-zero temperature results in the lethal disruption of cells due to formation of ice crystals and therefore they are not storable under condition suitable for orthodox seeds. Thus the term orthodox and recalcitrant have been used to describe the post harvest physiology of seeds. A category intermediate between orthodox and recalcitrant is also recognized in which seed survive desiccation but becomes damaged during dry storage at low temperature. *Ex-situ* conservation of seeds in seed banks in species producing recalcitrant and intermediate seeds is problematic for conservation of biodiversity.

*B. aristata* is one of the important medicinal plants of India as usd in Ayurvedic system of medicine (Biswas and Mukherjee 2003). Almost every part of this plant has some

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medicinal value. Its roots, stem, bark and fruits are used in many *ayurvedic* preparations and it also possesses antimicrobial activity (Musumeci *et al*. 2003). No information is available on seed morphology, dormancy, germination characteristics and seed storage behavior in *B. aristata* which is essential for storing the seeds in seed bank for germplasm conservation. Therefore, experiments were conducted with the objectives to study (i) seed morphology and anatomy (ii) effect of different temperatures on seed germinability and (iii) storage behaviour of seeds (with different moisture content) stored in different storage temperatures so as to examine the effect of moisture content on seed viability and desiccation and chilling tolerance. The seeds with varying (targeted) moisture content were stored in three different storage temperatures and seed germinability and seed viability was tested at three months interval up to 12 months. The topographical tetrazolium test was also standardized so as to study seed deterioration pattern in the embryos and to correlate the seed viability with seed germinability after desiccation and storage in three different storage temperatures.

## MATERIALS AND METHODS

Mature fruits of *Berberis aristata* were collected from Almora, Uttarakhand, India (latitude: 29°37' N, longitude 79°40' E, altitude: 1638 m above msl) and seeds were extracted from the fruits by pressing the seeds in water. Seeds were washed and dried under ambient laboratory condition during March-April 2009. Seed moisture content (mc) was determined in three replicates of 5g seeds each after drying for 17 hr in an oven at 103°C (ISTA 2008) and moisture content expressed as percentage of fresh weight. Due to heterogeneity in seed size, the lot was purified by gravity separator and air and screen cleaner machine and medium sized seeds which constitute the bulk are used for present studies while undersized and oversized seeds comprising about 5% and 2%, respectively were discarded.

Freshly extracted and dried seeds (100 × 4) having moisture content of 6.7% were germinated in 20, 25, and 30°C incubation temperatures using between paper method (rolled towel test) so as to determine the dormancy status, optimum temperature for germination and time taken for germination. Observations were taken on final per cent germination (normal seedlings) and seed vigour (as reflected by seedling length). A seed was considered germinated when all the essential structure of the seedlings were visible so as to differentiate between normal and abnormal seedlings. Seed viability tests (using topographical tetrazolium chloride test) were also standardized and conducted so as to correlate the viable seeds with germinable seeds, as a viable seed may not germinate due to dormancy. Seed morphology and anatomy was also studied.

Two hundred (50 × 4) seeds were soaked in water for 17 (±1) hr at 20ºC and imbibed seeds were cut transversely ¼ from the distal end (opposite to the stalk base), then transferred in 1 % of 2,3,5 triphenyl tetrazolium chloride solution and kept at 30ºC. It required 18 hr for complete staining of embryo. Then seeds were cut longitudinally through endosperm, and the embryo were exposed, washed and kept in water and examined for staining pattern using a stereoscopic microscope. The embryos were categorized as viable (embryos as well as surrounding endosperm completely stained), partly viable (incomplete staining of, i e less than half of the cotyledons and / or embryonic axis) and unstained (cotyledons and embryonic axis stained less than half or unstained). The data pertaining to staining pattern were correlated with standard germination test.

Seeds with 6.7% mc were again dried over silica gel in desiccators till they reached 5% moisture content. For preparing seeds with higher moisture content seeds were placed in saturated salt solution and repeatedly weighed till they reached the targeted moisture content following the protocol of Sun (2002) and seeds with targeted moisture content of 5, 7, 10 and 12% were prepared using the following formula

$$W_f = W_i\,[(100 - M_i) / (100 - M_f)]$$

where, W*f*, Final weight of sample; W*i*, initial weight of sample; M*i*, initial moisture content of sample; M*f*, final moisture content of sample.

Seeds having moisture content (mc) of 5, 7, 10 and 12% were tested for seed germinability (by conducting germination tests at 25°C) as well as seed viability (by conducting seed viability tests using topographical tetrazolium chloride tests) and subsequently stored in hermitically sealed containers in three storage temperatures, viz (a) ambient storage condition of the laboratory, where temperature varied from 15°C during winter to 37°C during summer, (b) at 15°C storage temperature in a temperature controlled room and (c) at –20°C in a refrigerator following the methodology given by Hong and Ellis (1996). Germination studies were conducted using 100 × 4 seeds by between paper methods (rolled towel) at 25°C incubation temperature as soon as seeds with targeted moisture contents were prepared and subsequently at three months interval up to 12 months. Seeds stored at –20°C and seeds with 5, 7, and 10% mc were kept at room temperature (20-25°C) with prevailing humidity for about 24 hr before putting for germination tests to prevent imbibitional damage (Ellis *et al.* 1990, Scande *et al*. 2001). Observations were taken on final per cent germination (normal seedlings), time taken for 50% germination and seed vigour (as reflected by seedling length). The seedlings and un-germinated seeds were classified as: normal seedlings, abnormal seedlings, fresh un-germinated seeds and dead seeds.

## RESULTS AND DISCUSSION

### *Seed morphology and anatomy*

The fruit of *B. aristata* is a berry, green when young and turn into purple black in colour at maturity. Seeds are

Table 1 Effect of different temperatures on per cent germination of seeds of *Berberis aristata*

| | Incubation temperature (°C) | | |
|---|---|---|---|
| | 20 | 25 | 30 |
| Germination (%) | 72(58.2) | 85(67.2) | 15(22.4) |
| CD | 4.234 | | |

*Values in parenthesis are the arc-sine values.

rhomboid in shape with 4.1 mm in length 2.7 mm in breadth and 3.6 $mm^3$ in diameter, testa ornamentation is reticulate (finely) with depression on ventral side, dark brown-black in colour, seed coat is thin and brown in colour. Thousand seed weight was 6.48g. The longitudinal section (LS) show a centrally placed spatulate embryo surrounded by endosperm. The endosperm is thin at the micropilar region comprising 2-3 layers. Embryo consisting of two large cotyledons (embryonic leaves) and with a prominent radicle (embryonic root). Hypocotyl is poorly differentiated. Embryo to seed ratio (E: S ratio) is low (0.5) as it fills middle of the seed volume. During the course of evolution of Angiosperms, the relative size of embryo has increased which resulted occurrence of non-dormant seeds.

*Effect of temperature on percentage germination*

Seeds obtained from freshly harvested fruits were germinated in different incubation temperatures, viz. constant temperature of 20, 25, and 30°C. Perusal of data in Table 1 reveals that seeds are non-dormant and 25°C incubation temperature was optimum as significantly less germination was observed at 20 and 30°C temperature respectively. The times taken for first and final count were 11 days and 17 days respectively.

*Effect of dehydration and hydration on seed germinability and seed viability*

Desiccation or hydration of seeds did not influence percent germination of seeds (Table 2), as no significant difference in germination or seed viability was observed due to dehydration or hydration. Perusal and co-relation of the data on seed germination and seed viability (Table 2) reveals

Table 2 Effect of hydration and dehydration data on seed viability and germinability in *Berberis aristata*

| | Moisture content (%) → | | | | |
|---|---|---|---|---|---|
| | 5 | 7 | 10 | 12 | Mean |
| Viability (%) | 85 (67.0) | 86 (68.7) | 82 (65.1) | 80 (63.4) | 83.2 (65.8) |
| Germi-nation(%) | 82 (65.1) | 85 (67.0) | 80 | 78 | 81.2 (64.3) |
| Mean | 83.5 (66.0) | 85.5 (67.9) | 81 (64.1) | 79 (62.6) | |

Correlation value between viable seed (%) and germination (%) is 0.963 among the four moisture content at ambient storage.

high coefficient of correlation (>0.9) between percent germination and per cent viability (completely stained embryo). The unstained embryo were correlated with dead seeds while partially stained embryo produced abnormal seedlings with root missing (radical unstained) or deformed cotyledons (cotyledons partially stained).

*Effect of desiccation, storage temperature and storage period on seed germinability*

Perusal of the data in Fig 1 and Table 3 reveals that percent mean germination was reduced during ambient storage compared to initial germination at the time of storage. Similarly germination during storage was adversely affected in seeds having higher moisture content compared to lower moisture content. However no significant difference in mean germination was observed in seeds having 5 and 7% moisture content. Seeds with 5% moisture content did not show significant reduction in germination up to 3 months of storage as germination at the time of storage was 82.3% which was reduced to 76.3% after 6 months in ambient storage. However no significant reduction in germination was recorded at 6, 9 and 12 months of storage in seeds with 5% mc. Seeds with 7% moisture content decline rapidly compared to seeds with 5% mc. As significant reduction in germination was observed at 6 months of storage and no significant reduction was observed from 6 months onwards. No significant difference in germination was recorded in seeds with 5 and 7 % moisture

Table 3 Effect of moisture content on germination percentage of *Berberis aristata* under ambient storage

| Moisture | Storage period in (months) | | | | | |
|---|---|---|---|---|---|---|
| content (%) ↓ | 0M | 3M | 6M | 9M | 12M | Mean |
| 5 | 82.3(65.155) | 80.6(63.936) | 76.3(60.887) | 75(59.979) | 74.3(59.575) | 77.7(61.906) |
| 7 | 84.6(67.038) | 77.6(61.789) | 77.6(61.789) | 77.3(61.567) | 75.6(60.438) | 78.42(62.524) |
| 10 | 79.6(63.173) | 60(50.801) | 53(46.703) | 35.3(36.378) | 12(20.249) | 47.98(43.461) |
| 12 | 77.6(61.774) | 42.6(40.726) | 35.6(36.621) | 32.3(34.603) | 3(9.876) | 38.22(36.720) |
| Mean | 81.02(64.285) | 65.2(54.313) | 60.47(51.500) | 54.97(48.132) | 41.22(37.535) | |

CD Moisture content (MC) : 1.85
Storage period (SP) : 2.07
MC*SP : 4.14

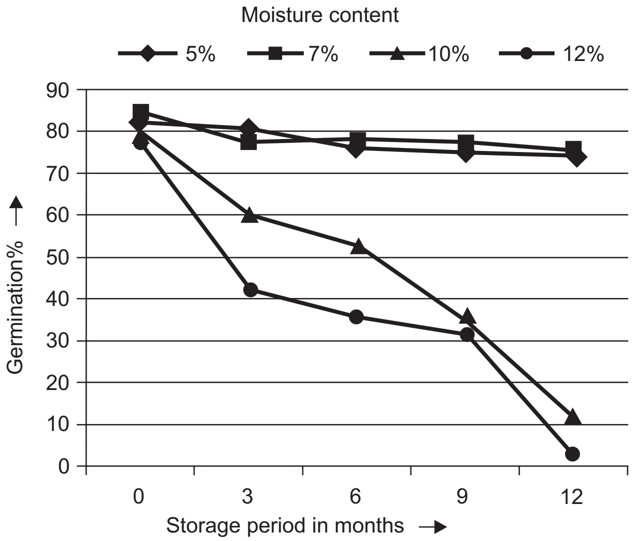


Fig 1 Effect of storage period and moisture content on seed germinability of *Berberis aristata* under ambient condition.

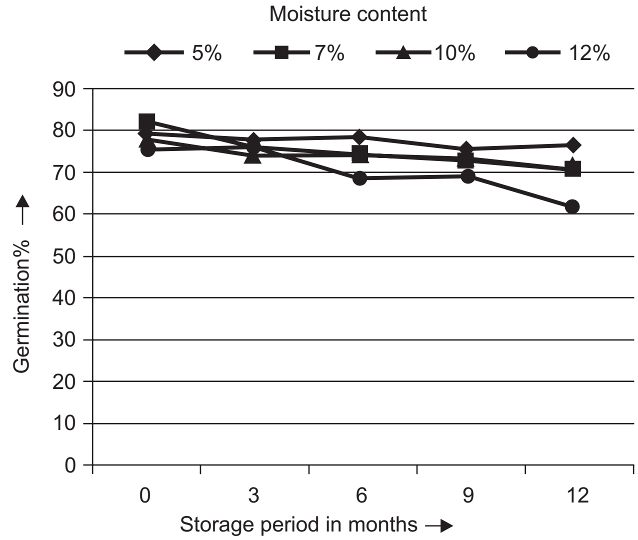


Fig 2 Effect of storage period and moisture content on seed germinability of *Berberis aristata* under storage at 15°C.

content from 6 to 12 months of storage. Decline in germination was much faster in seeds with having 10 and 12% mc as germination was 79.6 and 77.6 % at the time of storage which reduced to 12 and 3% respectively after 12 months in ambient condition.

In general decline in germination was less in seeds stored at 15ºC compared to ambient storage condition (Fig 1 and Table 3 and Fig 2 and Table 4). No significant reduction in germination was observed in seeds with 5 and 7% mc even after 12 months of storage. However significant reduction in germination was observed in seeds with 10 and 12% moisture content (Fig 2 and Table 4).

Storage of seeds at –20°C proved beneficial as the seeds were not only desiccation tolerant as well as chilling tolerant (Fig 3 and Table 5). No significant reduction in germination was observed after 12 months of storage as temperature and moisture content interaction was not significant. However slight decline in mean germination was observed after 12 months of storage. Germination in seeds with 12% mc declined from 77.6% to 67.3% after 12 months of storage at

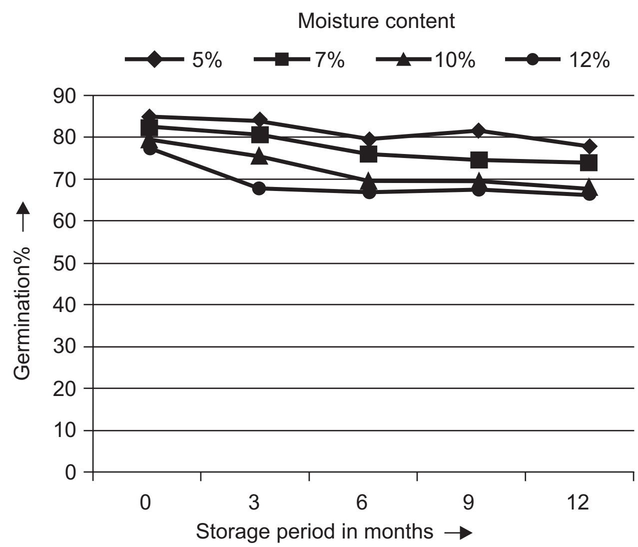


Fig 3 Effect of storage period and moisture content on seed germinability of *Berberis aristata* under storage at –20°C.

Table 4 Effect of moisture content on germination percentage of *Berberis aristata* under storage at 15ºC

| Moisture content (%) ↓ | Storage period in months 0M | 3M | 6M | 9M | 12M | Mean |
|---|---|---|---|---|---|---|
| 5 | 81.6(64.668) | 79.6(63.199) | 80.6(63.901) | 77.6(61.808) | 78.3(62.243) | 79.54(63.164) |
| 7 | 84.6(67.038) | 78.3(62.243) | 76.3(60.887) | 74.6(59.814) | 72.3(58.257) | 78.3(61.648) |
| 10 | 79.6(63.173) | 76(60.654) | 75.6(60.610) | 74.6(59.774) | 73.6(59.108) | 74.6(60.664) |
| 12 | 77.6(61.774) | 78(62.071) | 70(56.774) | 71(57.429) | 63(52.521) | 71.38(58.114) |
| Mean | 80.85(64.163) | 77.9(62.042) | 75.6(60.543) | 74.4(59.706) | 71.8(58.032) | |

CD Moisture content (MC) : 1.58
Storage period (SP) : 1.77
MC*SP : NS

Table 5 Effect of moisture content on germination percentage of *Berberis aristata* under storage at –20°C

| Moisture content (%) ↓ | Storage period (Months) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | Mean |
| 5 | 84.6(67.038) | 83.6(66.241) | 79.6(63.173) | 81.6(64.686) | 78(62.142) | 81.48(64.656) |
| 7 | 82.3(65.155) | 80.6(63.936) | 76.3(60.887) | 75(59.979) | 74.3(59.575) | 77.7(61.906) |
| 10 | 79.6(63.173) | 76(60.645) | 70.3(57.026) | 70.3(56.980) | 68.6(56.073) | 72.9(58.779) |
| 12 | 77.6(61.774) | 68.6(55.968) | 67.6(55.326) | 68.6(55.974) | 67.3(55.126) | 69.9(56.834) |
| Mean | 81.02(64.285) | 77.2(61.698) | 73.75(59.103) | 73.87(59.405) | 72.05(58.229) | |

CD Moisture content (MC): 1.78
Storage period (SP) :2.00
MC*SP : NS

–20°C, suggesting that higher moisture content ≥ 10% was having adverse effect on seed viability or seeds were chilling sensitive at higher mc.

Considering the above it can be concluded that seeds showed purely orthodox storage behavior and can be stored at freezing temperature after lowering its moisture content (to 5-7%) for a longer period of time.

Storage temperature and seed moisture content are the most important factors affecting seed longevity, with moisture content usually more influential than temperature. Roberts (1972) described the relationship of temperature and moisture content to the period of seed viability of certain crop species exhibiting orthodox storage behavior. Harrington (1972) proposed thumb rule and stated that the life of seed is halved (1) for each one percent increase in moisture content (2) for each 5°C increase in seed storage temperature. Although reduction in moisture content and storage temperature increases the life span of true orthodox seeds, 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, Vertucci *et al*. 1994). 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 (Probert and Hay 2000, Buitink and Hoekstra 2004). 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 (Buitink and Hoekstra 2004).When seed mc falls below a certain value, the cytoplasm become 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 (Buitink *et al*. 2000, 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. The ability of seeds to survive desiccation is an important functional trait and is an integral part of plant regeneration ecology as the overwhelming majority of spermatophytes (*c*. 92 %) have seeds that tolerate drying to low levels (Tweddle *et al*. 2003). Majority of species distributed in arid and semi-arid region produce orthodox seeds as seed desiccation sensitivity is largely confined to plants growing in humid and high rainfall regions and more common in non-pioneer evergreen rain forest trees and shrubs.

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## REFERENCES

Berjak P and Pammenter N W. 1997. Progress in understanding and manipulation of desiccation sensitive (recalcitrant) seeds. *Basic and Applied Aspects of Seed Biology*, pp 689–703. Ellis R H *et al*. (Eds). Kluwer, Dordrecht, The Netherland.

Berjak P and Pammenter N.W. 2003. Biology of recalcitrant seeds. *Encyclopedia of Applied Plant Sciences*, pp 56–62. Thomas B *et al.* (Eds). Elsevier, UK.

Biswas T K and Mukherjee B. 2003. Plant medicines of Indian origin for wound healing activity: a review. *International Journal of Lower Extremity Wounds* **2**(1): 25–39.

Buitink J and Hoekstra F A. 2004. Understanding and predicting optimal storage condition and longevity. *Seed Conservation: Turning Science into Practice*, pp 747–59. Smith R D *et al*. (Eds). The Royal Botanic Gardens, Kew, London.

Buitink J, Leprince O, Hemminga M A and Hoekstra F A. 2000. Molecular mobility in the cytoplasm: an approach to describe and predict life span of dry germplasm. *Proceedings of the National Academy of Science,* USA **97**: 2 385–90.

Ellis R H. 1998. Longevity of seeds stored hermetically at low moisture content. *Seed Science Research* (Supplement No. 1) **8**: 9–10.

Ellis R H and Roberts E H. 1980. Improved equations for the prediction of seed longevity. *Annals of Botany* **45**: 13–30.

Ellis R H and Hong T D. 2007. Seed longevity-moisture content relationship in hermetic and open storage. *Seed Science & Technology* **35**: 423–31.

Ellis R H, Hong T D and Roberts E H. 1988. A low moisture content limit to logarithmic relations between seed moisture content and longevity. *Annals of Botany* **61**: 405–8.

Ellis R H, Hong T D and Roberts E H. 1990. Effect of moisture content and method of rehydration on the susceptibility of pea seeds to imbibitional damage. *Seed Science & Technology* **18**: 131–7.

Engelmann F and Engels J M M. 2002. Technologies and strategies for *ex situ* conservation. *Managing Plant Genetic Diversity*, pp 89–103. Engels J M M *et al.* (Eds). CABI Publishing, Wallingford, UK.

Harrington J F. 1972. Seed storage longevity. *(in) Seed Biology, 3*, pp 145–245, Kozlowski T T (Ed).Academic Press, New York.

Hong T D and Ellis R H. 1996. *A protocol to determine seed storage behaviour*. IPGRI Technical Bulletin No. 1, IPGRI, Rome, Italy.

Hong T D, Ellis R H, Astley D, Pinnerar A E, Groot S P C, Kraak H L**.** 2005. Survival and vigour of ultra-dry seeds after ten years of hermetic storage. *Seed Science and Technology* **33**: 449–60.

ISTA. 2008. International Rules for Seed Testing, Edition 2008. International Seed Testing Association (ISTA), Bassersdorf, Switzerland.

Linington S H and Pritchard H W. 2001. Genebanks. *Encyclopedia of Biodiversity,* Vol 3, pp 165–181. Levin S (Ed). Academic Press, New York.

Musumeci R, Speciale A, Costanzo R, Annino A, Tagusa S, Rapisarda A, Pappalardo M S and Iauk L. 2003. *Berberis aetnensis* C. Presl: antimicrobial properties and interaction with ciprofloxacin. *International Journal of Antimicrobial Agents* **22**(1): 48–53.

Nagel M and Borner A. 2010. The longevity of crop seeds stored under ambient condition. *Seed Science Research* **20:** 1–12.

Pritchard H W and Dickie J B. 2004. Predicting seed longevity: the use and abuse of seed viability equations. *Seed Conservation: Turning Science into Practice*, pp 653–722. Smith R D *et al*. (Eds). Royal Botanic Gardens Kew, London.

Probert R J. and Hay F R 2000. Keeping seed alive. *Seed Technology and its Biological Basis,* pp 375–407. Academic Press, London.

Roberts E H. 1972. Storage environment and control of viability. *Viability of Seeds,* pp 14–58. Roberts E H (Ed). Chapman & Hall, London.

Roberts E H. 1973. Predicting the storage life of seeds. *Seed Scence. & Technology* **1**: 499–514.

Sacandé M, Golovina E A, van Aelst A C and Hoekstra F A. 2001. Viability loss of neem (*Azadirachta indica*) seeds associated with membrane phase behaviour. *Journal of Experimental Botany* **52**: 919–31.

Sun W Q. 2002 Methods for the study of water relations under desiccation stress. *Desiccation and Survival in Plants Drying Without Dying*, pp: 48–91.Black M, and Prichard H W (Eds). CABI.

Tweddle J C, Dickie J B, Baskin C C and Baskin J M. 2003. Ecological aspects of seed desiccation sensitivity. *Journal of Ecology* **91**: 294–304.

Vertucci C W, Roos E E and Crane J. 1994. Theoretical basis of protocols for seed storage III. Optimum moisture contents of pea seeds stored in different temperatures. *Annals of Botany* **74**: 531–40.