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Seed dormancy, germination and seed storage in henna (*Lawsonia inermis*)

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ABSTRACT

Henna (*Lawsonia inermis* L.) is an important commercial plant of India grown mainly for its leaves used for dying hair, skin etc. The seeds of henna are small typically pyramidal, endospermic with a linear embryo. The freshly harvested seeds show endogenous non-deep physiological dormancy and pre-treatment of seeds, viz. leaching, chilling, priming, GA_3 and KNO_3 co-application reduced dormancy. The physiological dormancy was transitory and disappeared during storage due to after-ripening. Seed storage studies revealed that seeds with 5 and 7 % moisture content did not show significant reduction in seed longevity up to 24 months in ambient storage in comparison to seeds with higher moisture content. Seed deterioration was slow at 15 and –20°C storage temperatures. The seeds are desiccation as well as chilling tolerant, therefore, exhibit orthodox seed storage behaviour which makes them ideal for *ex-situ* conservation in seed banks for long term storage.

Key words: Dormancy, Germination, Henna, *Lawsonia inermis*, Seed storage, Seed longevity

Henna (*Lawsonia inermis* L., syn. *L. alba* Lam. belonging to family *Lytharaceae*) is a much branched glabrous shrub and native of sub-tropical region of Asia and Africa. It is commonly cultivated in the western parts of India, Pakistan, Morocco, Yemen, Iran, Sudan and Libya. Presently, Pali district of Rajasthan is the most heavily cultivated henna production area in India, with over 100 henna processors operating in Sojat city alone (http://www.underutilized-species.org/species/brochure/Henna_.pdf). Henna has many traditional and commercial uses, the most common being as a dye for hair, skin and fingernails, a dye and preservative for leather and cloth (Chaudhary *et al.* 2010, Siva 2007). The plant was reported to have analgesic, antibacterial, antifungal, anti-parasitic and many similar properties (Babu and Subhasree 2009).

Henna is also cultivated as a hedge plant and as live fencing to protect the crop fields from grazing animals (Chaudhury *et al*. 2005). Henna is the third most important medicinal and aromatic plant of India after *Plantago ovata* L. (isabgol) and *Cassia angustifolia* Vahl. (senna) in term of export value (Parihar *et al*. 2009). Information on seed dormancy and germination characteristics, moisture content for safe storage of seeds for short term as well as long term seed conservation is not available which is essentially required for growing the crop, storage of seeds and also for seed quality assurance etc. Therefore, studies were conducted with the objectives to know (i) seed morphology and anatomy, (ii) kind of dormancy, duration of dormancy and time required for complete germination in freshly harvested and six month stored seeds in different temperatures and effect of seed pre-treatments on dormancy and germination and (iii) effect of seed moisture content, storage temperatures and storage period on viability of seeds so as to understand the effect of seed moisture and storage temperature on seed longevity and also to know desiccation and chilling sensitivity for conservation of seeds in seed banks.

MATERIALS AND METHODS

Mature globose fruits (which turns green to brown at maturity) of henna were collected from Indian Agricultural Research Institute, New Delhi (latitude: 28° 38' 23"N, longitude: 77° 9' 27" E, altitude: 228.6 m above msl) grown as a hedge plant during October 2007 and seeds were obtained by drying the fruits, which split open after drying. The seeds were dried in the laboratory for about 8-10 days and moisture content in the seeds was determined (103°C for 17 hr) following ISTA (ISTA 2006), till the moisture content was stabilized in the prevailing relative humidity and temperature. Seeds were cleaned using a seed blower to remove the inert matter and about one kg of seed was processed using specific gravity separator. Undersized and oversized seeds comprising about 8 and 3% by weight respectively were discarded in order to obtain a homogenous seed lot. The main seed fraction with 8.1 moisture content having 1 000 seed weight of 1.324 g was stored in plastic bottles for subsequent studies.

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Germination studies were conducted using top of the paper method (TP) because of smaller seed size using petri-dishes (12 cm diameter) with Whatman germination papers. The seeds (100 seeds in four replications) were arranged equidistantly on the surface of the paper. The covered petri-dishes were placed in the incubator having desired temperature with light during the day. Preliminary studies conducted earlier with seeds obtained from commercial source revealed that seeds are positively photoblastic (require light for germination) and leaching of seeds in water enhanced germination (Parihar *et al*. 2009, Parihar 2012). Therefore, seeds were pre-treated before germination in different temperature regimes, viz. 20, 25, 30, 35, 20/35 ^{o}C (higher temperature for 6 hr in a day). The treatments given were: T-0; (no treatment), T-1 and T-2; leaching (seeds were leached in running water for 24 hr and 48 hr, respectively), T-3; GA_3 treatment (seeds soaked in gibberellic acid (GA_3 500 ppm) for overnight (17 hr): T-4; KNO_3 (seeds soaked in 0.2% of KNO_3 solution for overnight, T-5; priming (seed priming, seeds were soaked in water at 20^{0}C for 17 hr and dried back to original moisture content), T-6; pre-chilling (moistened seeds were kept for two days at 5 ^{o}C before putting for germination in petri-dishes in respective temperatures). 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 per cent 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. Per cent germination was expressed in nearest whole number as per standard practice used in seed trade (ISTA 2006). 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.

The processed and homogenous seed lot having 8.1% moisture content (mc) was again dehydrated / moistened using saturated salt solutions (Gold and Hay 2008) in desiccators and seeds with targeted mc of 5, 7, 8, 10, 12 and 15% 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 six targeted mc were stored in hermetically sealed containers in three storage temperatures, viz. (a) ambient laboratory condition- where temperature varied from 15^{o}C in winter to 37°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 TP method at 20/35°C before storage (after preparing the seeds with desires targeted moisture content) and after storage at three months interval up to 24 months following Hong and Ellis (1996) (the experimental design consisted of six moisture contents, three storage temperatures and nine storage periods). Seed vigour as reflected by seedling length was determined on the first count (5th day) using 20 seedlings per replication selected randomly. Light was provided for 6 hr in all the germinating temperatures as germination was adversely affected under dark condition. Seeds with 5 and 7 % mc were humidified for 24 hr in 90 % RH in desiccators (maintained by using saturated salt 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

Seed morphology and anatomy

Seeds of henna are small (measuring 1.94-2.63 mm from micropylar to chalazal end and widest at the chalazal end from 1.94 to 2.67 mm), typically pyramidal with 3-4 vertical surfaces, endospermic with linear embryo located towards micropylar end. The micropylar endosperm is 2-3 layered while chalazal endosperm is 7-8 layered. The testa is yellowish brown and cells are rich in lawsone (secondary metabolite responsible for colouring) content. Germination is epigeal and level of embryo differentiation (in seed) is low. Embryo-seed ratio (the relative size of embryo within the seed) is also low ranging from 0.3 to 0.4 as the endosperm fills the remaining portion of seed. During the course of seed evolution of angiosperms, the embryo – seed ratio increased with no or very little endosperm and the highly evolved seed type is represented by a foliate axile embryo type with a high level of embryo differentiation. Therefore, seeds of henna represent a less advanced stage of evolution with low embryo seed ratio and low level of embryo differentiation (Linkies *et al.* 2010).

Seed dormancy, germination and time taken for germination in fresh and six months stored seeds: Perusal of data in Table 1, reveals that mean germination was significantly higher ($P \geq 0.05$) in treated seeds in comparison to control (T-0; no seed pre-treatment) in fresh seeds. The effect of temperature was also significant as mean germination was significantly higher at 35 and 20/35°C compared to 20, 25 and 30°C. However, no significant difference in per cent germination was recorded in 35 and 20/35°C suggesting presence of physiological dormancy with initial higher temperature requirement. Time taken for germination was up to 21 days in lower temperature while it was eight days in 35 and 20/35°C. This kind of dormancy with initial high temperature requirement has

Table 1 Effect of seed pre-treatments on germination (%) of fresh seeds in different temperatures

Treatment	Incubation temperatures (°C)					
	20 °C	25 °C	30 °C	35 °C	20/35 °C	Mean
T0 (control)	71.0 (57.42)*	72.0 (58.05)	74.0(59.34)	93.0(74.66)	95.0(77.08)	81.0 (64.16)
T1	80.0(63.44)	82.0(64.90)	85.0(67.21)	94.0(75.82)	96.0(78.48)	87.4 (69.21)
T2	83.0(65.65)	83.0(65.65)	86.0(68.03)	92.0(73.57)	94.0(75.82)	87.6(69.38)
T3	81.0(64.16)	80.0(63.44)	84.0(66.42)	94.0(75.82)	93.0(74.66)	86.4(68.36)
T4	79.0(62.72)	79.0(62.72)	82.0(64.90)	95.0(77.08)	95.0(77.08)	86.0(68.03)
T5	82.0(64.90)	82.0(64.90)	84.0(66.42)	94.0(75.82)	93.0(74.66)	87.0(68.87)
T6	83.0(65.65)	83.0(65.66)	82.0(64.90)	93.0(74.66)	95.0(77.08)	87.2 (68.95)
Mean	79.8 (63.29)	80.1(66.50)	82.4(65.20)	93.5(75.23)	94.4 (76.06)	
CD (P = 0.05)	Treatments 2.10, temperature 1.92, treatment × temp. 3.45					

*Figures in parenthesis are arc sine √percentage transformation.

Table 2 Effect of seed pre-treatments on germination (%) of six month stored seeds in different temperatures

Treatment	Incubation temperatures (°C)					
	20	25	30	35	20/35	Mean
T0	83.0 (65.65)*	91.0(72.54)	96.0(78.48)	93.0(74.66)	95.0(77.08)	91.6(73.16)
T1	91.0(72.54)	93.0(74.66)	94.0(75.82)	94.0(75.82)	96.0(78.48)	93.6(75.35)
T2	93.0(74.66)	91.0(72.54)	91.0(72.54)	92.0(73.57)	94.0(75.82)	92.2(73.78)
T3	95.0(77.08)	95.0(77.08)	95.0(77.08)	94.0(75.82)	93.0(74.66)	94.4(76.31)
T4	93.0(74.66)	94.0(74.82)	93.0(74.66)	95.0(77.08)	95.0(77.08)	94(75.82)
T5	94.0(75.82)	92.0(73.57)	91.0(72.54)	94.0(75.82)	93.0(74.66)	92.8(74.44)
T6	93.0(74.66)	93.0(74.66)	94.0(75.82)	93.0(74.66)	95.0(77.08)	93.6(75.35)
Mean	91.7 (73.28)	92.7(74.32)	93.4(75.15)	93.5(75.23)	94.4(76.31)	
CD (P = 0.05)	Treatments 2.28, temperature 1.93, treatments × temp 5.11					

*Figures in parenthesis are arc sine ‘‘percentage transformation.

been classified as endogenous non-deep physiological dormancy, type 1 (Baskin and Baskin 2004), which is considered to be the most widely accepted eco-physiological classification of seed dormancy (Linkies *et al.* 2010). Germination of freshly harvested and untreated seeds ranged from 71-74 % in lower temperatures, viz. 20, 25 and 30°C, while germination was > 90% in higher/alternating temperatures, i.e. 35°C and 35/20°C (Table 1). The optimum temperature for germination broadens after c. 6 months of dry storage under ambient condition since, no significant difference in per cent germination was observed after 6 months of storage in different incubation temperatures (except at 20°C) as the optimum temperature for germination broadens, suggesting that dormancy was overcome due to after ripening (Table 2). This type of dormancy is often transitory and disappears during dry storage. The time required for germination was also reduced after six months storage as compared to fresh seeds. After-ripening of seeds during storage is associated with a loss of dormancy due to change in the physiological status of seed, which takes place at a seed moisture content between 8 to 15% (Probert 2000, Bezin *et al.* 2011). The precise mechanisms underlying dormancy breaking by after-ripening remain elusive but have been correlated with changes in gene expression, enzyme activity, and hormone accumulation, suggesting that biological processes such as transcription and translation can occur in dry seeds (Finch-Savage *et al*. 2007, Finkelstein *et al.* 2008). Mature seeds are usually described as dry, although they contain some water, enough to support gene expression.

Time required for germination was 7-8 days in 35 and 35/20°C in fresh as well as 6 months stored seeds, while in fresh seeds time taken for germination was up to 21 days in lower temperatures again suggesting the presence of non-deep endogenous physiological dormancy. Physiological dormancy is the most common dormancy class found in the seeds of angiosperms and believed to be regulated by the existence of growth inhibitors, promoters or the balance between them (Leubner-Metzger 2003, Nonogaki 2006). Among the compounds that act to induce seed dormancy is abscisic acid (ABA), which appears to play an important role, both by its presence in seed or by sensitivity of the embryo to its action. The use of ABA deficient mutants and inhibitors of ABA synthesis has been helpful in demonstrating the importance of ABA in dormancy induction of several crops (Finch-Savage and Leubner-Metzger 2006). Leaching, GA_3 and KNO_3 co-application, chilling and priming has also been found helpful in overcoming the dormancy by modifying the balance of compounds that inhibit or promote germination in seed (McDonald 2000). Small seeds require

Table 3 Effect of seed moisture content (mc) and storage period on % germination of seeds stored under ambient condition

Storage periods (months)	Moisture content in seeds (%)						Mean
	5	7	8	10	12	15	
0	92.0 (73.57)*	92.0(73.57)	94.0(75.83)	93.0(74.66)	93.0(74.66)	93.0(74.66)	92.8(74.44)
3	94.0(75.82)	93.0(74.66)	91.0(72.54)	85.0(67.21)	88.0(69.73)	23.0(28.66)	79.0(62.72)
6	90.0(71.56)	94.0(75.82)	93.0(74.66)	81.0(64.16)	62.0(51.94)		70.0(56.79)
9	93.0(74.66)	90.0(71.56)	92.0(73.57)	84.0(66.42)	42.0(40.40)		66.8(54.82)
12	90.071.56)	93.0(74.66)	89.0(70.63)	54.0(57.29)	43.0(40.98)		61.5(51.65)
15	91.0(72.54)	91.0(72.54)	84.0(66.42)	41.0(39.82)	12.0(20.27)		53.1(46.78)
18	89.0(70.63)	88.0(69.73)	71.0(57.42)	42.0(40.40)			48.3(44.03)
21	92.0(73.57)	88.0(69.73)	63.0(52.53)	18.0(25.10)			43.5(41.27)
24	93.0(74.66)	91.0(72.54)	63.0(52.53)				41.1(39.87)
Mean	91.5(73.05)	91.1(72.64)	82.2(65.05)	55.3(48.04)	37.7(37.88)	12.8(20.96)	
CD (P = 0.05)	Storage period 1.49, mc 1.21, storage period × mc 3.65						

*Figures in parenthesis are arc sine √percentage transformation

light for germination to avoid germination when they are too deep in soil or under plant shadows that could compete with the seedlings giving it little or no chance to survive (Geneve 2003). Light mediated induction of germination in seeds is governed by phytochrome, which implies that red light induces germination and far-red light inhibit it. It was determined that *Pfr*, the red absorbing or activated form of phytochrome induces germination by promoting GA synthesis (Leubner-Metzger 2003). In addition to light, temperature fluctuation is another signal, for a seed regarding its relative position in the surrounding vegetation. The temperature fluctuations during the day and night are lower deep in the soil and in the middle of abundant vegetation, than near the soil surface or in a gap without surrounding vegetation. This variation may explain why seed germination in henna is favored by alternating temperature compared to constant temperature. Changes in hormone sensitivity have also been suggested as a possible mechanism mediating induction of germination by alternating temperatures (Geneve 2003). Seed dormancy control, germination timings in response to seasons, plays an important role in evolution and adaptation to climatic changes (Forbis *et al.* 2002). Germination timings may strongly influence the rate at which species can expand their range and may play an important role in determining survival or extinction during climate change.

Effect of seed moisture content, storage temperatures and storage periods on seed viability and vigour: Perusal of data in Table 3 to 8 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. Perusal of data in Table 3 and 6 reveals that no significant reduction in germination and vigour was observed in seeds with moisture contents of 5 and 7% under ambient storage up to 24 months. Seeds with 8.0 moisture content also did not show significant reduction in germination and vigour up to 12 months storage suggesting that seeds up to 8% mc can be safely stored up to one year in ambient storage condition with no loss of viability and vigour. Loss of viability and vigour was comparatively slow when seeds were stored at 15°C (Table 4,7) and seeds with 15% moisture content could retain viability with 20 % germination up to 24 months of storage, while seeds with mc 5, 7 and 8% did not show significant reduction in

Table 4 Effect of seed moisture content (mc) and storage period on % germination of seeds (stored at 15°C).

Storage periods (months)	Moisture content in seeds (%)						Mean
	5%	7%	8%	10%	12%	15%	
0	94(75.82)*	95(77.08)	94(75.82)	93(74.66)	93(74.66)	93(74.66)	93.6(75.36)
3	94(75.82)	90(71.56)	88(69.73)	91(72.54)	88(69.73)	88(69.73)	70.3(56.98)
6	93(74.66)	94(75.82)	93(74.66)	94(75.82)	91(72.54)	81(64.16)	91.0(72.54)
9	93(74.66)	90(71.56)	92(73.67)	95(77.08)	86(68.03)	41(39.82)	82.8(65.90)
12	90(71.56)	91(72.54)	90(71.56)	85(67.55)	70(56.79)	38(38.06)	68.0(55.55)
15	91(72.54)	91(72.54)	93(74.66)	81(64.16)	68(55.55)	35(36.27)	76.5(61.00)
18	90(71.56)	89(70.63)	91(72.54)	82(64.90)	52(46.15)	21(27.28)	90.3(71.85)
21	90(71.56)	93(74.66)	92(73.57)	80(63.44)	51(45.57)	21(27.28)	78.3(62.24)
24	93(74.66)	93(74.66)	89(70.63)	75(60.00)	48(43.85)	18(25.10)	70.0(56.78)
Mean	92.0(73.57)	91.7(73.26)	91.3(72.84)	88(69.73)	77.8(57.92)	49(44.43)	
CD (P = 0.05)	Storage period 2.66, mc 2.17, storage period × mc 6.51						

*Figures in parenthesis are arc sine ‘‘percentage transformation.

Table 5 Effect of seed moisture content (mc) and storage period on % germination of seeds (stored at -20 °C)

Storage periods	Moisture content in seeds (%)						Mean
	5%	7%	8%	10%	12%	15%	
0	91.0(72.54)	91.0(72.54)	93.0(74.66)	93.0(74.66)	93.0(74.66)	89.0(70.63)	91.6 (73.15)
3	93.0(74.66)	94(75.82)	91(72.54)	91(72.54)	90(71.56)	92(73.57)	91.8(73.36)
6	89(70.63)	93(74.66)	90(71.56)	90(71.56)	91(72.54)	94(75.82)	91.1(72.64)
9	92(73.57)	92(73.57)	93(74.66)	94(75.82)	89(70.63)	93(74.66)	92.1(72.64)
12	90(71.56)	90(71.56)	91(72.54)	90(71.56)	93(74.66)	66(54.33)	86.6(68.53)
15	93(74.66)	90(71.56)	92(73.57)	92(73.57)	90(71.56)	65(53.73)	87.0(68.87)
18	94(75.82)	94(75.82)	90(71.56)	90(71.56)	92(73.57)	52(46.15)	85.3(67.45)
21	92(73.57)	93(74.66)	93(74.66)	89(70.63)	80(63.44)	52(46.15)	83.1(65.73)
24	91(72.54)	91(72.54)	95(77.08)	93(74.66)	81(64.16)	50(45.0)	83.5(66.03)
Mean	91.6(73.15)	92(73.57)	92(73.57)	91.3(72.84)	88.7(70.36)	72.5(58.37)	
CD (P = 0.05)	Storage period 1.31, mc 1.07, storage period × mc 3.24						

germination and vigour up to 24 months in 15°C storage temperature (Table 4,7). Storing of seeds at –20°C could retain the viability and vigour in seeds having higher moisture content as no reduction in percent germination and seedling length was observed with seed having mc 5, 7, 8 and 10%, while significant reduction in germination and vigour was recorded in seeds with 12 and 15% mc after 21 and 12 months of storage respectively (Table 5,8).

Storage temperature and seed moisture content are the most important factors affecting seed longevity, with moisture content usually more influential than temperature. Owing to intricate relationship between storage temperature and seed moisture content, neither can be discussed separately. Roberts (1972) described the relationship of temperature and moisture content to the period of seed viability of certain crop species. Harrington (1972) proposed thumb rule and stated that the life of seed is halved for each one per cent increase in moisture content and/or for each 5°C increase in seed storage temperature. The range of moisture content and temperature for the rule was considered 5-14% and 0-50°C, respectively. 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 and Hoekstra 2004). The critical moisture content for storage depends on storage temperature and chemical composition of seeds. The critical moisture content of henna seeds under ambient storage condition (up to 24 months) appears to be 7 % as no significant loss in viability and vigour was observed between seeds of 5 and 7% mc (Table 3,6). The critical mc increased to 8% at 15°C (Table 4, 7) and to 10% at –20°C (Table 6,8). Reduction in germination and vigour at –20°C in seeds having 12 and 15 % mc is attributed to chilling injury of seeds due to higher moisture content in orthodox seeds.

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

Table 6 Effect of seed moisture content (%) and storage period on seedling length (mm) of seeds stored under ambient condition

Storage period (months)	Moisture content (mc) in seeds						Mean
	5%	7%	8%	10%	12%	15%	
0	162.2	154.5	166.2	153.1	159.8	157.2	158.8
3	165.5	160.2	168.0	154.0	142.0	116.0	150.9
6	166.2	162.0	165.0	164.0	121.6		129.8
9	167.2	166.8	167.8	162.0	121.0		130.8
12	160.8	165.0	166.0	152.8	110		125.7
15	157.8	166.0	153.7	148.0	90.2		119.2
18	156.2	160.0	142.0	145.6			100.6
21	154.8	156.8	131.7	121.5			94.1
24	158.2	151.8	121.0				71.8
Mean	160.9	160.3	153.4	133.4	82.7	30.3	
CD (P=0.05)	Storage period 4.62, moisture content 3.77, storage period × mc 11.32						

Table 7 Effect of seed moisture content and storage period on seedling length (mm) of seeds stored at 15 °C

Storage periods (months)	Moisture content (mc) in seeds						
	5%	7%	8%	10%	12%	15%	
0	163.8	160.8	166.2	152.8	164.3	158.0	160.9
3	162.6	160.0	165.0	154.0	160.0	162.0	160.6
6	165.6	162.2	164.5	164.0	160.0	160.0	162.7
9	165.0	167.0	168.0	161.8	155.0	158.0	162.4
12	161.0	165.0	166.0	165.0	154.0	150.0	160.1
15	157.8	165.7	160.7	165.5	158.0	148.0	159.2
18	156.0	160.0	160.0	150.2	128.0	122.0	146.0
21	154.7	157.8	155.5	144.6	132.0	122.0	144.4
24	158.0	154.0	153.0	142.0	121.0	121.0	141.5
Mean	160.5	161.3	162.1	155.5	148.0	144.5	
CD (P=0.05)	Storage period 4.24, mc 3.52, storage period × mc 12.52						

Table 8 Effect of seed moisture content and storage period on seedling length (mm) of seeds stored at –20°C

Storage period (months)	Moisture content (mc) in seeds						Mean
	5%	7%	8%	10%	12%	15%	
0	160.2	161.6	165.8	161.6	164.2	165.3	163.0
3	162.6	160.0	165.0	157.0	160.0	162.2	161.1
6	165.8	162.0	164.5	161.2	160.0	159.5	162.2
9	164.5	167.2	168.0	162.0	154.8	148.0	160.7
12	161.3	165.0	168.2	165.3	154.0	115.0	154.8
15	158.0	166.0	161.0	166.0	157.6	110.0	153.1
18	156.0	160.0	160.5	166.5	147.3	90.0	146.7
21	155.0	157.6	156.0	157.0	140.0	87.0	142.1
24	158.0	154.0	158.0	158.0	141.6	90.0	143.2
Mean	160.1	161.4	163.0	161.6	153.2	125.2	
CD (P = 0.05)	Storage period 3.26, mc 4.32, storage period × mc 13.32						

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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