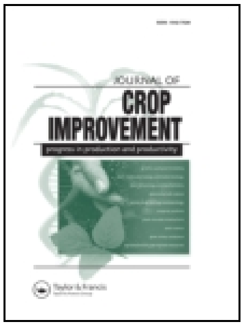

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Differential Longevity of Castor (*Ricinus communis* L.) Germplasm Conserved Under Uncontrolled Storage Conditions Across Extended Periods

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*Castor researchers and seed producers mostly store seed under uncontrolled conditions. The ability of castor (*Ricinus communis *L.) seed to remain viable across extended periods of storage under such conditions has not been widely investigated, although this is an important issue for maintaining seed lots. Seed longevity of 2,961 castor accessions stored for <1–19 years under ambient conditions was investigated to know how castor seed loses vigor in long-term storage and to determine the genetic variability for seed longevity. The results showed that castor seed retained 40% germination even after 19 years of storage, and no accession lost germination completely up to four years of storage under ambient conditions. All the accessions maintained 100% germination for one year, but from second year onwards differential germination percentage (10%–100%) was observed among accessions stored for the same duration; some accessions stored for 11–14 years maintained 80%–100% germination, whereas some stored for three years had <50% germination. Therefore, we concluded that it would be safe to store seed of any castor genotype for one year after harvest under ambient conditions, but beyond that the genotypic response needs to be considered for maintaining high germination percentage (80%–100%). The information generated would be useful to researchers and seed producers in properly maintaining seed lots.*

KEYWORDS *castor seed, genetic variation, germination, oil content, seed weight*

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INTRODUCTION

Castor (*Ricinus communis* L.) is an important industrial oilseed crop (Anjani 2014). It can be grown in tropical, sub-tropical, and temperate regions on marginal lands. Its seed is orthodox in nature that can be maintained satisfactorily *ex situ* over the long term in appropriate environments. Castor seed lots with less than 70–80% germination are rejected for certification (ESA 2012; Trivedi and Gunasekaran 2013). Long longevity of seed is a desirable trait as it reduces regeneration frequency and allows long-term *ex situ* conservation in gene banks and open-storage facilities. Conserving seed in storage modules and monitoring of seed longevity are expensive. Maintenance of seed germinability is a basic condition for successful conservation of germplasm and for storage of commercial and breeder's seed. Castor researchers, seed producers, and farmers store seed under uncontrolled conditions. Viability of seed decreases rapidly under uncontrolled conditions with increasing seed age. The length of time seed can remain viable under a given set of storage conditions indicates seed longevity. There are no reports on longevity of castor seed when stored across extended periods under uncontrolled ambient conditions and on existence of genetic variation for longevity in castor. A preliminary investigation was carried out using the seeds of 2,961 castor germplasm accessions stored under ambient conditions for <1–19 years to learn how castor seed loses vigor in long-term storage and to determine the genetic variability for seed longevity.

MATERIALS AND METHODS

The seeds of 2,961 castor germplasm accessions stored under ambient conditions for <1–19 years were planted on 6 July 2011 at the research farm of the Directorate of Oilseeds Research (DOR), Hyderabad, India, where the soil was characterized as an Alfisol. Fresh seed of all accessions, prior to storing in a storage room, was sundried for 3–4 weeks until seed moisture content reached around 7% ± 0.5 and then packed in thin wax-coated brown paper bags. The bags were stored in steel racks placed in a closed room. During the extended periods of storage under ambient conditions, the lowest minimum temperature was 10°C and the highest maximum temperature was 42°C, while relative humidity ranged between 37% and 85%. Seed moisture content during storage was monitored; it did not increase over the initial content of 7% ± 0.5. Seeds were free from insects and fungi during storage.

Ten seeds of each accession were sown in a single row of 6 m length with 90 cm spacing between rows and 45 cm between plants. Percent emergence of an accession in the field represented percent germination, which in

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turn represented percent longevity of that accession. As it is difficult to record seed germination inside the soil when planted in the field, seedling emergence was used to indicate vigor of germinated seed to emerge out of the soil. Genebank curators, seed producers, and breeders are more interested in field emergence of seed than germination alone after prolonged storage under ambient conditions for maintenance of experimental seed lots and their multiplication.

Meteorological data for the year 2011–12 were obtained from an automatic weather station located at the research farm of DOR, Hyderabad, India. Around 42 mm rainfall was received in the planting week, and 33, 6.4, and 73.6 mm rainfall was received in the succeeding three weeks, respectively. There was no water-logging and soil crusting in any part of the field during and after seedling emergence. The maximum temperature varied between 27° and 32°C and the minimum temperature between 21 and 23°C at the experimental farm from the planting week to one month after planting. The number of seedlings that emerged in the field was recorded during 10th to 20th day after planting. Seedling establishment in the field for each accession was recorded 30 days after planting. Seed oil content and 100-seed weight were recorded for each accession prior to storage. Seed oil content was analyzed prior to storage using nuclear magnetic resonance equipment (MQA-7005, Oxford Scientific Instruments). The Pearson correlation coefficient (r), mean values, and range of 100-seed weight and oil content were calculated using INDOSTAT statistical software (Indostat Services, Hyderabad, India). Backward stepwise multiple linear regression analysis was done using SAS Enterprise Guide 4.3, (SAS Institute Inc 2010).

RESULTS

Seeds of accessions took 7 to 10 days to emerge from the soil. No seedling abnormalities were observed in the field irrespective of seed storage duration. Differential ability of accessions to remain viable under uncontrolled storage environment was clearly evident. Wide genetic variation was observed for longevity among castor accessions conserved for same duration as well as for different durations (Table 1). The 64 accessions stored for less than one year and the 178 accessions stored for one year exhibited 100% germination. Among the 2719 accessions stored for different durations, ranging from two to 19 years, 2,091 accessions maintained 10% to 100% germination. No accession lost complete germination when stored for four years. There was complete germination loss in about 6% and 11% of the accessions stored for five and six years, respectively. About 22% to 25% of accessions stored for 7 to 10 years lost germination completely. Fifty percent of the accessions stored for 11 years lost germination, whereas none of the accessions stored for 12 years lost germination. About 64% of accessions stored for 13 years, and 37% of

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TABLE 1 Germination percentage of 2,961 castor germplasm accessions after storing for different durations under uncontrolled conditions and seed weight and oil content of these accessions

Duration of storage (years)	Total number of accessions	100-seed weight (g)	Oil content (%)	Number of accessions: Germination (%) 0	10	20	30	40	50	60	70	80	90	100
19	1	21–30	43	–			–	1	–	–	–	–	–	–
14	35	21–30	44–53	13	2	2	2	1	1	–	4	7	1	2
13	28	11–20	45–47	8	–	–	–	1	–	–	–	1	–	–
		21–30	37–54	10	–	–	–	–	–	1	1	2	1	3
12	20	21–30	46–48	19	–	–	–	–	–	–	–	1	–	–
11	16	11–20	49–52	2	–	–	–	–	–	–	–	–	–	–
		21–30	45–52	6	–	–	–	–	1	–	–	2	–	5
10	430	1–10	43	–	–	–	1	–	–	–	–	–	–	–
		11–20	38–55	15	1	2	4	1	2	2	–	5	1	28
		21–30	34–55	62	3	16	18	17	5	11	20	62	12	77
		31–40	30–55	18	3	3	2	–	2	2	1	3	–	11
		41–50	42–53	7	–	–	–	1	–	1	1	3	–	2
		51–60	47–49	1	–	–	–	–	–	–	–	1	1	1
		81–90	48	1	–	–	–	–	–	–	–	–	–	–
9	243	1–10	48–49.5	2	–	–	–	–	–	–	–	–	–	–
		11–20	28–38	19	1	1	1	3	1	3	1	7	–	14
		21–30	34–54	32	5	8	3	8	4	5	4	22	7	49
		31–40	28–54	7	2	1	2	–	–	3	–	6	–	11
		41–50	41.1–54	2	–	1	–	1	–	–	–	3	–	3
		51–60	48	–	–	–	–	–	–	–	–	1	–	–
8	1252	1–10	49.4–49.8	2	3	10	4	1	1	3	1	–	–	–
		11–20	39–54	39	23	42	37	32	38	25	20	6	3	2
		21–30	30–55	245	82	140	94	126	80	47	43	117	25	97
		31–40	40–54	29	2	4	1	5	3	4	3	3	1	11
		41–50	41–53	6	1	–	–	–	–	–	–	3	–	4
		51–60	49	–	–	–	–	–	–	–	–	–	–	1

(*Continued*)

TABLE 1 (Continued)

Duration of storage (years)	Total number of accessions	100-seed weight (g)	Oil content (%)	Number of accessions										
				Germination (%)										
				0	10	20	30	40	50	60	70	80	90	100
7	230	1–10	44.2–50.5	1	–	–	2	1	–	–	–	–	–	–
		11–20	33.8–54	13	1	9	6	4	8	2	4	1	–	13
		21–30	28.4–55	26	–	14	10	10	1	4	9	7	3	30
		31–40	41.8–52.2	9	1	2	1	2	1	4	1	2	–	13
		41–50	40.4–52	3	–	2	2	–	–	–	–	–	–	6
		51–60	51.2	–	–	1	–	–	–	–	–	–	–	–
		61–70	50.15	–	–	–	–	–	–	–	–	–	–	1
6	161	11–20	41–50.8	–	–	2	–	1	–	–	–	–	–	7
		21–30	44–53	17	–	1	3	–	16	6	4	2	–	76
		31–40	47–54	1	–	–	–	3	3	–	1	3	–	10
		41–50	48	–	–	1	–	1	–	1	–	–	–	1
		51–60	47.2	–	–	–	–	–	–	–	–	–	1	–
5	226	11–20	42–53	–	–	–	–	–	1	3	2	2	1	19
		21–30	40–54	12	1	10	4	3	2	8	4	23	10	88
		31–40	39.4–53.7	2	1	1	1	–	1	2	1	4	2	10
		41–50	43.8–51	–	–	–	–	–	–	1	–	–	2	3
		51–60	49	1	–	–	–	–	–	–	–	–	–	–
		71–80	45	–	–	–	–	–	–	–	–	–	–	1
4	20	11–20	47	–	–	–	–	–	–	–	–	–	–	1
		21–30	45–52	–	–	–	–	–	–	2	4	4	2	3
		31–40	43.9–53	–	–	–	–	–	–	–				4
3	37	11–20	45–54	–	3	–	1	–	–	–	1	–	–	3
		21–30	38–53	–	3	2	1	–	–	2	2	1	–	12
		31–40	46–48	–	–	1	–	–	–	1	–	1	–	1
		41–50	48–51	–	–	–	–	–	–	–	–	–	–	2
2	20	11–20	43.2–51	–	–	–	–	–	2	–	–	–	–	3
		21–30	32–51	–	–	–	–	–	–	3	4	–	–	5

		41–50	42–51	–	–	–	–	–	–	–	–	–	1	2
1	178	11–20	28.1–53	–	–	–	–	–	–	–	–	–	–	25
		21–30	32.6–55	–	–	–	–	–	–	–	–	–	–	89
		31–40	43.8–55	–	–	–	–	–	–	–	–	–	–	44
		41–50	32–54	–	–	–	–	–	–	–	–	–	–	11
		51–60	41.52–48	–	–	–	–	–	–	–	–	–	–	5
		61–70	48	–	–	–	–	–	–	–	–	–	–	1
		71–80	48	–	–	–	–	–	–	–	–	–	–	1
<1	64	1–10	46.4	–	–	–	–	–	–	–	–	–	–	1
		11–20	28.1–54	–	–	–	–	–	–	–	–	–	–	27
		21–30	41–55	–	–	–	–	–	–	–	–	–	–	6
		31–40	41.3–53	–	–	–	–	–	–	–	–	–	–	18
		41–50	42–51	–	–	–	–	–	–	–	–	–	–	6
		51–60	46–49	–	–	–	–	–	–	–	–	–	–	2
		71–80	48–50	–	–	–	–	–	–	–	–	–	–	2
		81–90	45	–	–	–	–	–	–	–	–	–	–	1

Mean: 52.68% (germination %), 26.74 g (100-seed weight), 47.99% (oil content).
SD: 42.19 (germination %), 6.67 (100-seed weight), 3.34 (oil content).
SEm± = 0.77 (germination %), 0.12 (100-seed weight), 0.06 (oil content).

accessions stored for 14 years lost germination completely. One accession (RG-2089) stored for 19 years maintained 40% germination and 25 accessions stored for 11 to 14 years maintained high germination percentage (80%–100%) (Table 2).

All of the castor accessions that were tested exhibited substantial variation for 100-seed weight, with a range from 7 to 87 g and an average of 27 g. Substantial variation was also observed among the accessions for oil content, which ranged from 26% to 55%, with a mean of 48% (Table 1). There was little or no association between seed weight and oil content (r = 0.12**). The backward stepwise multiple linear regression analysis revealed no apparent effect of seed weight ($R^2 = 0.1934$) and seed oil content ($R^2 = 0.1937$) on seed germination of accessions stored for different durations. The analysis also showed a significant weak effect of storage duration on germination ($R^2 = 0.199$).

TABLE 2 Castor germplasm accessions having high germination percentage after storing for 11–14 years under uncontrolled storage conditions

Accession	Storage duration (years)	Germination percentage
RG 29	14	80
RG 602	14	80
RG 691	14	80
RG 854	14	90
RG 860	14	80
RG 911	14	80
RG 1298	14	100
RG 1389–1	14	80
RG 1456	14	80
RG 1525	14	80
RG 2068	14	100
RG 1663	13	80
RG 1668–1	13	80
RG 1695	13	90
RG 1969	13	100
RG 2065	13	80
RG 2210	13	100
RG 2067	12	80
RG 97	11	80
RG 659	11	80
RG 817	11	100
RG 931	11	100
RG 1594	11	100
RG 2296	11	100
RG 2350	11	100
Mean		88.0
SD		9.38

DISCUSSION

Seed moisture content and storage temperature are the most important non-genetic factors affecting seed longevity. Sundried seed of castor contains around 7% moisture content (Akande et al. 2012). Seed moisture content between 6% and 10% is ideal for storing castor seed (Savy Filho 2005). Mendes et al. (2010) found that the accelerated aging at 45°C and 100% RH for 48, 72, and 96 hours caused excessive deterioration of castor seeds. In this study, the seed moisture content during storage was below 10% and the temperature was below 45°C. Pandey and Radhamani (2006) reported that castor seed maintained viability well under long-term storage conditions of -20°C but lost viability significantly at 10°C, and maintained 65%–70% germination after storage for five years at ambient temperature. They also observed a significant decline in seed viability in small castor seeds stored at ambient temperature for five years based on observations on two samples each in bold and small category seed. However, in the present study, no correlation between seed weight and longevity was observed. This was definitely because of use of a comparatively very large sample size than that used by Pandey and Radhamani (2006). No correlation between seed mass and longevity was also reported in many crop species (Pritchard and Dickie 2003; Walters, Wheeler, and Grotenhuis 2005). Seed oil content was also not correlated with seed longevity under ambient storage conditions in the present study. Such findings were also reported in other crops (Pritchard and Dickie 2003; Walters, Wheeler, and Grotenhuis 2005; Probert, Daws, and Hay 2009). These reports suggested that lipid composition might not have played an important role in determining longevity but further analysis would be necessary to demonstrate it.

Lago et al. (1979) had found that post-harvest dormancy was not a constraint in castor. Machado et al. (2010) reported that 9.3% of seeds were dormant just after harvest, which decreased to 5.5% when stored for 12 months at room temperature, and the seeds harvested from different order of racemes on a castor plant did not differ in germination. In the present investigation also, dormancy was not a constraint since germination was 100% when seeds were planted four months after harvest. Bewley and Black (1994) demonstrated that germination was not a constraint if developing castor seed was collected 50 days after pollination (DAP); they also demonstrated the ability of excised seed to germinate by 25 DAP, if excised seed were desiccated. This suggests that seed drying prior to sowing was more important than dormancy in castor.

Castor seed germination is mostly dependent on soil temperature, soil moisture availability, and salinity (Zhou et al. 2010; Severino et al. 2012). The reported optimum temperature for castor seed germination was 22° to 25°C, the minimum was 10° to 15°C, and the maximum was 36°C (Atsmon 1966; Cheema et al. 2008). The minimum (21–23°C) and maximum (27–32°C) temperatures in the experimental plots from the time of planting to one month after planting were within the ideal limits of temperature identified for castor seed germination, thus ruling out adverse effect of temperature on germination. Soil moisture and salinity also did not affect germination, as the soils of the experimental plots were non-saline since the average electrical conductivity of soils was 0.22 ds/m; the experimental plots received 42 mm rainfall during planting week and 33 mm in the following week and one irrigation 15 days after planting. The complete establishment of all germinated seeds in the field was indicative of good health and quality of seeds. Seed aging did not affect seedling emergence and seedling health. Sufficient quantity of fully mature seed was harvested from each plant.

High seed longevity is a desirable trait for conservation of seeds for desired periods. Seeds of some species under natural conditions can remain viable for very long periods, extending up to decades (Roberts 1972). The present investigation revealed that castor seed retained 100% germination after one year of storage under ambient conditions irrespective of the genetic constitution, and some genotypes could retain 100% germinability even after 14 years of storage and remained viable (40%) after 19 years. Linseed retained an average 90% germination after eight years of storage, which dropped to around 10% after 13 years of storage under ambient conditions (Nagel and Borner 2010), whereas germination declined to 77%–78% in sunflower (Balesevic-Tubic et al. 2010), 80% in safflower, and 56%–70% in soybean (Vijay and Dadlani 2003; Balesevic-Tubic et al. 2010) after one year of storage under ambient conditions; and after 13 months of storage under ambient conditions, germination was 84% in sesame, 66% in niger, 78% in groundnut, and 88% in groundnut after 13 months of storage under ambient condition (Kurdikeri et al. 1996). Yadav et al. (2013) reported decline of germination to 70%–73% after two years of storage in Indian mustard (*Brassica juncea*) seeds collected from various branches.

Although reduction in percent germination was observed from second year onwards in the present investigation, no accession lost germination ability completely up to four years. Later, the percentage of accessions losing complete germination increased with increased storage duration until 12 years (7%–95%) (R^2 = 0.5125), but such linear trend was not observed afterwards in the data (Figure 1). Similarly, the percentage of accessions retaining 80%–100% germination did not show a consistent linear trend (R^2 = 0.482) with duration of storage (Figure 2). This may be because of differential ability of accessions to longevity or may be because of unequal number of accessions stored in each year; the number of accessions stored from 5 to 10 years was more than 100, whereas it was quite less (1–35) beyond 10 years. However, the differential longevity of castor accessions stored for the same duration could be attributed in part to differences in genetic constitution of the accessions since the accessions concurrently suffered identical fluctuations in storage conditions. Seed longevity variation within a species has been

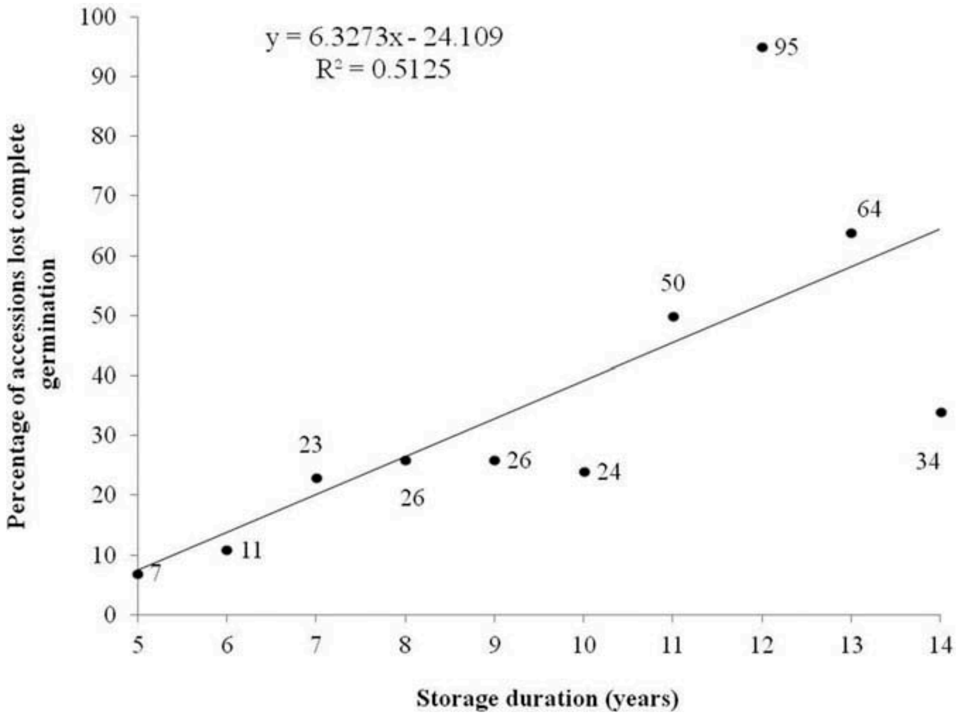

FIGURE 1 Percentage of castor germplasm accessions lost complete germination under uncontrolled storage conditions across years.

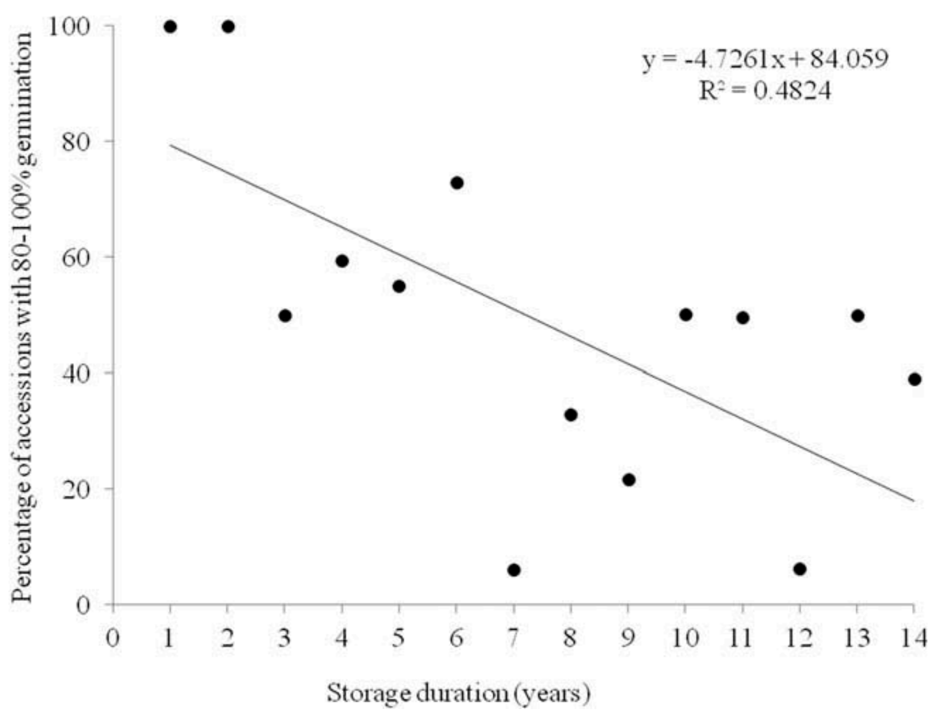

FIGURE 2 Percentage of castor germplasm accessions retained 80–100% germination under uncontrolled storage conditions across years.

demonstrated in barley (Hernandez, Carballo, and Hernandez 1991), sorghum (Sastri, Upadhyaya, and Gowda 2008), maize (Revilla et al. 2006), and in many other crops under controlled and uncontrolled (Nagel and Borner 2010) conditions. Nagel et al. (2011) suggested that the differential longevity reported in 42 *Brassica napus* accessions, stored in *ex situ* at the German *ex situ* genebank at IPK, Gatersleben, could be in part genetically determined. Recently, genome-wide association mapping and biochemical markers in barley have revealed that seed aging and longevity are intricately affected by genetic background and developmental and environmental conditions (Nagel et al. 2015). Differential longevity among accessions was indicative of existence of natural allelic variation for longevity. QTLs for seed longevity have been identified after natural aging in Arabidopsis (Bentsink et al. 2000; Clerkx et al. 2004), Brassica napus (Nagel et al. 2011), lettuce (*Lactuca sativa*) (Schwember and Bradford 2010), maize (Revilla et al. 2009), and rice (*Oryza sativa*) (Sasaki, Fukuta, and Sato 2005) and under artificial aging in Arabidopsis (Bentsink et al. 2000), rice (Miura et al. 2002) and wheat (Landjeva, Lohwasser, and Borner 2009). Sugliani et al. (2009) identified natural modifiers for better seed longevity in Arabidopsis accessions. The DNA damage and genome instability that occur in seeds during long-term storage were considered to cause the reduced germination after aging. The deferentially responded accessions for longevity when stored under same storage conditions suggests existence of natural allelic variation in castor for longevity. The natural genetic variants with long longevity (13, 14, 19 years) under ambient conditions would be useful to identify better alleles for long longevity in castor. Natural modifiers for better seed longevity were identified in Arabidopsis accessions (Sugliani et al. 2009), which could improve seed longevity in mutants (*abi3-5, lec-3*) that quickly lose seed viability due to impaired seed maturation.

The results demonstrated that some castor genotypes had ability for long longevity (11–19 years), while some lost viability by fifth year, but all maintained viability for four years of storage under ambient conditions. The information generated would be useful to castor breeders, seed producers, and germplasm curators in deciding the storage duration of castor seed lots.

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