STORAGE BEHAVIOUR OF *JATROPHA CURCAS* SEEDS

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DUONG TH, SHEN JL, LUANGVIRIYASAENG V, HA HT & PINYOPUSARERK K. 2013. Storage behaviour of *Jatropha curcas* seeds. The storage behaviour of *Jatropha curcas* seeds was studied. Seeds collected from China, Papua New Guinea and Vietnam were tested for germination capacity after storing at three different temperature regimes: room temperature (28 °C), cool room (4 °C) and freezer (-23 °C) for up to 24 months. Seed moisture contents, which were determined prior to and after storage, were within the range 8–10%. None of the seeds stored at room temperature germinated after 12 months while those stored at 4 and -23 °C remained viable. Seed germination deteriorated substantially from 12 to 24 months. The decrease (85–98%) was more pronounced at -23 °C. Cool room storage at 4 °C was most suitable for storage of *J. curcas* seeds with more than 50% of seeds remaining viable after 24 months. The seed moisture contents of 8–10% appeared too high for deep freeze storage of *Jatropha* seeds. The results of this study confirmed the orthodox storage behaviour of *J. curcas* seeds.

Keywords: Longevity, storage temperature, storage duration, seed moisture content, germination capacity

DUONG TH, SHEN JL, LUANGVIRIYASAENG V, HA HT & PINYOPUSARERK K. 2013. Kelakuan penyimpanan biji benih *Jatropha curcas*. Kelakuan penyimpanan biji benih *Jatropha curcas* dikaji. Keupayaan percambahan biji benih daripada negara China, Papua New Guinea dan Vietnam diuji selepas biji benih disimpan pada tiga suhu berlainan: suhu bilik (28 °C), bilik sejuk (4 °C) dan peti ais (-23 °C) sehingga 24 bulan. Kandungan lembapan biji benih sebelum dan selepas penyimpanan berjulat antara 8% hingga 10%. Biji benih yang disimpan pada suhu bilik tidak bercambah selepas 12 bulan tetapi biji benih yang disimpan pada 4 °C dan -23 °C dapat hidup. Percambahan anak benih berkurangan dengan banyak dari 12 bulan hingga 24 bulan. Pengurangan (85% hingga 98%) adalah lebih ketara pada -23 °C. Penyimpanan di bilik sejuk pada 4 °C paling sesuai untuk biji benih *J. curcas* kerana lebih daripada 50% biji benih dapat hidup selepas 24 bulan. Kandungan lembapan biji benih sebanyak 8% hingga 10% nampaknya terlalu tinggi bagi penyimpanan biji benih *Jatropha* di dalam peti ais. Keputusan kajian mengesahkan bahawa kelakuan penyimpanan biji benih *J. curcas* adalah ortodoks.

INTRODUCTION

Jatropha curcas (Euphorbiaceae) commonly called physic nut is a deciduous, multipurpose shrub or small tree distributed naturally in Mexico and elsewhere in Central America. It has been introduced and has become naturalised in many parts of the tropical and subtropical regions of the world (Heller 1996). Its seed, leaf and bark have medicinal values. It is commonly grown in South-East Asia as hedges to protect gardens and fields from animals. In recent years, it has achieved prominence as a candidate species for biodiesel production. This species is very adaptable to a wide range of soil and climate, and grows well without special nutrition (Patil & Singh 1991). It thrives on marginal land where many other species fail. It can be propagated from seed or cutting of stem or branch (Heller 1996). Propagation from seed, however, is considered better for establishment of long-lived plantations for seed-oil production. *Jatropha* produces large quantities of oil-seed within 2–3 years after planting. Seed yields vary considerably from 1.7–2.2 t ha^{-1} on poor barren soils to 3.9–7.5 t ha^{-1} on normal fertile soils (Kant & Wu 2011). Seed yield of 2 t ha^{-1} is considered not a viable investment.

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The economic importance of *J. curcas* has generated worldwide interest in plantation establishment for production of oil-seeds. A global market study on *J. curcas* in 2004 revealed that there were more than 1 mil ha of plantation established in Asia, Africa and Latin America (Euler & Gorriz 2004). There are at least 2 mil ha already under cultivation in China for the production of oil-seed (Fairless 2007). The scale of *Jatropha* plantation area is expected to increase to 12.8 mil ha by 2015, of which 70% will be situated in Asia (Euler & Gorriz 2004). Meeting such large-scale planting targets will require a very substantial quantity of seeds annually.

Nursery managers often have to carry over seed stock from one growing season to the next. The ability of a seed to retain viability is, therefore, an important factor for successful production of planting stock. This is because rapid loss of viability affects seedling production and jeopardises planting programmes. Seed longevity is influenced by biological and environmental factors. Based on the storage behaviour of seed, three categories have been identified: orthodox, intermediate and recalcitrant (Roberts 1973, Ellis et al. 1990a, Hong & Ellis 1996). Orthodox seeds can be dried to low moisture contents (2–6%) without damage and long-term storage is possible in cool and dry environments. Recalcitrant seeds, in contrast, can only be stored at high moisture contents close to their fully imbibed condition for short periods at moderate temperatures well above freezing. The intermediate category exhibits behaviour between orthodox and recalcitrant seeds, i.e. seeds can be dried to 7–10% moisture content but, even so, they generally lose viability rapidly when stored at low temperatures.

Effects of storage temperature on longevity of seeds have been documented for many Euphorbiaceae species (Ellis et al. 1985). Seed storage behaviour of *J. curcas* is, however, not well understood. *Jatropha curcas* seeds are oily and, therefore, are not expected to store well (Ellis et al. 1990b). Parreño-de Guzman and Aquino (2009) dried *J. curcas* seeds to 4.0–9.5% moisture content and stored them in sealed packets at 20 and 0 °C for up to 1 year and found that moisture content had more effect on germination over time than storage temperature. They recommended that *J. curcas* seeds be dried to 4–5% moisture content, sealed in moisture-proof containers to minimise viability loss. As a species of the Euphorbiaceae, the storage behaviour of *J. curcas* seed should be orthodox (Heller 1996). A report, however, claims that *J. curcas* seeds are recalcitrant (Green Africa Foundation 2007).

Following a wide-ranging provenance seed collection of *J. curcas* in 2009 from more than 40 sources in countries coordinated by CSIRO Australian Tree Seed Centre and the Forest Science Institute of Vietnam (FSIV) (Shen et al. 2012), an experiment was conducted to determine the optimum storage temperature using seeds from subsamples of three of those provenances. This paper reports the germination of *J. curcas* seed stored at three temperature regimes (28, 4 and -23 °C) over a period of 24 months. Such information will help in understanding the storage behaviour of *J. curcas* seeds and increase the efficiency of seed procurement activities for plantation establishment and domestication programmes.

MATERIALS AND METHODS

Seed material

Bulked seedlots of 10–20 parents of *J. curcas* seeds collected in Yunnan, China (CN144); Morobe, Papua New Guinea (PG136) and Sonla, Vietnam (VN119) were used in the study. Following the wide-ranging field collections in early 2009, the seeds were air-freighted to the FSIV seed laboratory in Hanoi and stored in sealed containers in a cool room at 4 ± 2 °C. A subsample of 100 seeds each from all seedlots was used to determine the morphological parameters of seeds, namely, length, width, thickness and weight. Moisture contents were determined for all seedlots from the wide-ranging collection and found to be within the range 8–10%. Seed length, width and thickness were measured using digital veneer callipers. Weight of 100 seeds was measured using an electronic balance. Of the three sources, seeds from Papua New Guinea were largest and heaviest (Table 1). Seeds from China were slightly smaller than those from Vietnam.

Storage conditions

Three storage temperature regimes were used: room temperature (average 28 °C), refrigerated cool room (4 ± 2 °C) and freezer (-23 °C). The freezer was a self-defrosting type. The seeds of each source were placed in multiple sealed aluminium foil packets, each containing 100 seeds to allow four replicates of germination tests to be conducted at regular intervals. The seed packets representing each source were randomly subjected to the three storage temperatures. Only packets that needed to be tested were randomly taken from storage.

Seed moisture content

Moisture content (MC) of the seeds was determined before the commencement of experiment and 24 months after storage at different temperatures. For each seedlot, the MC was determined gravimetrically on 15 seeds (3 replicates of 5) which were oven dried for 17 hours at 105 °C.

Seed germination tests

All germination tests were conducted in a nursery at the FSIV seed laboratory. An initial germination test was carried out on 19 May 2009. Two more germination tests were carried out after storage for 12 and 24 months on 19 May 2010 and 19 May 2011 respectively. The seeds were taken from storage temperatures and soaked in water for 6 hours before sowing. All germination tests were carried out with four replicates of 25 seeds sown in trays filled with sand which was kept moist by spraying with water twice a day. The seeds were pressed down horizontally to the surface level. Germination was monitored every day for 30 days. Germination in *J. curcas* was epigeal. Seeds were considered germinated when the seed coat had shed from the hypocotyl. From our previous unpublished observations, these germinants would develop into normal seedlings.

Statistical analysis

Data were analysed at plot mean level using analysis of variance (ANOVA) procedure in GenStat Discovery (Williams et al. 2002, Payne et al. 2003). A factorial arrangement of 3 seed sources, 3 storage temperatures and 2 storage durations was carried out to determine significant differences in germinated seeds between treatments. The ANOVA for seed MC was based on seed sources and storage conditions. Least significant differences were used to compare the treatment means. No transformation of the percentage data was required since the equal variance assumption was not violated.

Due to marked differences between seedlots in the initial germination percentage, we examined the magnitude of loss of viability due to storage temperatures by comparing the per cent decrease in seed germination relative to their initial germination percentage. The following equation was used to compute relative decrease in germination for each storage time (duration):

Relative decrease in germination (%) =

$$\frac{Gi - Gt}{Gi} \times 100$$

Table 1 Characteristics of *Jatropha curcas* seeds (mean values) used in seed storage study

Seed source	Code	Length (mm)	Width (mm)	Thickness (mm)	100-seed weight (g)
Yunnan, China	CN144	17.0 ± 1.2	10.0 ± 0.8	7.9 ± 0.6	62.4 ± 4.1
Morobe, Papua New Guinea	PG136	18.6 ± 1.1	11.1 ± 0.7	8.9 ± 0.5	79.7 ± 5.9
Sonla, Vietnam	VN119	17.6 ± 0.9	10.7 ± 0.7	8.5 ± 0.7	65.5 ± 3.3

where Gi = initial germination and Gt = germination of stored seed at time t.

RESULTS

Seed moisture content

Initial MC differed significantly between seed sources. Moisture content of CN144 at 8.8% was significantly less ($p < 0.001$) than those of PG136 (9.7%) and VN119 (9.9%) (Tables 2 and 3). After 24 months of storage, the three seed sources followed a similar trend of declining MC under all three storage temperatures: lowest in CN144 and highest in VN119. Overall the seed MC after 24 months of storage was within the range 8–10%. The MC of VN119 seeds stored at 28 and -23 °C remained fairly stable at about 10%.

There was significant interaction between seed source and storage temperature (Table 2), resulting in a lack of consistent pattern of variation in the seed MC across storage temperature. In CN144 seeds, the MC increased, though not significantly, with decreasing storage temperature. In the PG136 and VN119 seeds, MC was significantly lowest at 4 °C compared with MC at 28 and -23 °C (Table 3).

Table 2 Summary for analysis of variance for testing significant difference in seed moisture content

Source of variation	Df	F value	p
Seed source (SS)	2	49.22	< 0.001
Storage temperature (ST)	2	9.22	< 0.01
SS × ST	4	2.86	< 0.05
Error	22		

Df = degree of freedom, p = probability

Germination of stored seeds

Highly significant differences in germination of stored seeds were observed between different seed sources, storage temperatures and storage durations (Table 4). Initial germination percentage (Table 5) was found to differ considerably between the three seedlots (50–93%). Complete loss of viability was observed for all seedlots stored at 28 °C after 12 months. However seeds remained viable at low temperatures (4 and -23 °C) over the same storage duration. CN144 seeds maintained almost the same level of germination percentage (60% at 4 °C and 57% at -23 °C) as the initial germination (61%). VN119 seeds had 33 and 24% germination at 4 and -23 °C respectively, representing a decrease of 34 and 52% from the initial germination. PG136 seeds maintained high germination at -23 °C losing only 9% of initial germination but more at 4 °C (26%).

Table 3 Seed moisture contents (%) of *Jatropha curcas* before and after storage for 24 months at different temperature regimes

Seed source	Initial MC	24-month MC		
		28 °C	4 °C	-23 °C
CN144	8.8	8.0	8.3	8.5
PG136	9.7	9.6	8.8	9.2
VN119	9.9	10.1	9.0	10.0

Least significance differences of means (5%): seed source (SS) = 0.28, storage temperature (ST) = 0.33, SS × ST = 0.57; MC = moisture content

Table 4 Analysis of variance for germination and decrease relative to initial germination with regard to seed source, storage temperature, storage duration and their interactions over 24 months of storage

Source of variation	Df	F value	p
Percentage germination			
Seed source (SS)	2	29.23	0.001
Storage temperature (ST)	2	178.79	0.001
Storage duration (SD)	1	123.49	0.001
SS × ST	4	7.65	0.001
SS × SD	2	14.52	0.001
ST × SD	2	57.65	0.001
SS × ST × SD	4	8.47	0.001
Error	51		
Percentage decrease relative to initial germination			
SS	2	5.18	0.01
ST	2	171.76	0.001
SD	1	103.45	0.001
SS × ST	4	3.38	0.05
SS × SD	2	6.34	0.01
ST × SD	2	49.69	0.001
SS × ST × SD	4	2.64	0.05
Error	51		

Df = degree of freedom, p = probability

Germination of the stored seeds declined substantially from 12 till 24 months and the decrease was more pronounced in seeds stored at -23 °C. While seeds stored at -23 °C lost 85–98% of initial germination, those stored at 4 °C still maintained more than 50% of initial germination (Table 5). Overall, Vietnamese seed VN119 maintained the best longevity at 4 °C storage temperature. The per cent decrease from the original germination at 24 months was almost the same as that at 12 months.

DISCUSSION

The initial germination of *J. curcas* seeds from China, Papua New Guinea and Vietnam varied considerably, though not statistically analysed. This is not uncommon in most plant species. Some of this variation can be attributed to genetic origin and environment but much of it is caused by seed collection and handling. The seeds in our study were collected by agencies in different countries and, therefore, some different practices in the collection and handling were to be expected. Seeds of higher initial germination (93%) from Papua New Guinea were less resistant to deterioration in storage than those of lower initial germination from China (61%) and Vietnam (50%). In fact, seeds from Vietnam showed the least deterioration after 2 years of storage at 4 °C. Seed MC was not expected to have an impact on the germination percentage of seeds stored under the same temperature regime. The MC, though different between seedlots and storage temperatures, was found to be within the range 8–10% before and after storage.

Storability of seeds is determined by the level of deterioration in germination capacity. Our results indicated that the viability of *J. curcas* seeds was markedly influenced by storage temperature and storage duration. Seeds kept at room temperature of 28 °C for 12 months failed to germinate while those stored at 4 and -23 °C retained good viability. Extending the storage duration from 12 till 24 months resulted in a sharp drop in the germination percentage with the decline being more pronounced at -23 °C than at 4 °C. This pattern of loss in viability during storage was similar for all three seedlots. Cool room (4 °C) proved to be most suitable for storage of *J. curcas* seeds with more than half of the stored seeds remaining viable after 24 months in storage. Greater loss of seed viability under deep freeze storage compared with cool room storage has been reported for species such as *Carica papaya* (Ellis et al. 1991) and *Vaccinium membranaceum*

Table 5 Germination (%) of *Jatropha curcas* stored under different temperatures and durations

Seed source	Initial germination	28 °C		4 °C		-23 °C	
		12 mo	24 mo	12 mo	24 mo	12 mo	24 mo
CN144	61	0 (100)	0 (100)	60 (7)	32 (48)	57 (8)	9 (85)
PG136	93	0 (100)	0 (100)	66 (26)	49 (47)	85 (9)	2 (98)
VN119	50	0 (100)	0 (100)	33 (34)	32 (36)	24 (52)	2 (96)

Values in parentheses indicate per cent decrease relative to initial germination; mo = month

Least significance differences of means (5% level)	Per cent germination	Per cent decrease
Seed source (SS)	4.9	7.3
Storage temperature (ST)	4.9	7.3
Storage duration (SD)	4.0	4.0
SS × ST	8.5	12.6
SS × SD	6.9	10.3
ST × SD	6.9	10.3
SS × ST x SD	12.0	17.8

(Shafii & Barney 2001). The types of freezer used in those studies are not known. Unlike biomedical freezer, temperature in self-defrosting freezer fluctuates considerably. Loss of germination capacity of seeds stored at fluctuating subzero temperatures could be a result of ice desiccation injury. The seeds in our study were stored in a self-defrosting freezer, which may not be ideal.

Safe level of seed MC to avoid damage from deep freeze storage at -20 °C is 12.5–13.5% for cereal species and lower MC is necessary for species with oily seeds (Hong & Ellis 1996). Some suggested 6–8% as the maximum MC for long-term storage of oily seeds (Hayma 2003). *Jatropha curcas* seeds contain oil up to 40% of seed weight (Achten et al. 2007). Thus, the 8–10% MC of seeds in this study appears too high for safe storage in deep freeze condition. Further study of the effects of seed MC on seed storage behaviour of *J. curcas* at subzero temperature is warranted.

The storage behaviour of *J. curcas* seeds is considered to be orthodox based on the report by Parreño-de Guzman and Aquino (2009) that seeds remain viable with germination above 90% once dried to 4–5% MC and stored at 0 °C after 12 months. The results of this study where 8–10% MC seeds were viable after 24 months in 4 °C storage conditions supported this hypothesis.

CONCLUSIONS

Our study confirms that the seed storage behaviour of *J. curcas* is orthodox. We demonstrated the importance of storage temperature on seed viability, and that cool room (4 °C) storage in air-tight containers was most suitable. Room temperature of 28 °C contributed to accelerating deterioration and loss of seed viability within 12 months of storage. Both cool room (4 °C) and freezer (-23 °C) storage conditions were satisfactory in maintaining seed viability for 12 months but seeds lost nearly all germination capacity after 24 months at subzero temperatures. The seed MC of *J. curcas* was 8–10% which appeared to be beyond the safe level for subzero storage of oily seeds.

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