

## Desiccation, storage and germination of *Genipa americana* seeds

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### **Abstract**

Germination temperature dependency, tolerance to desiccation and sensitivity to exposure to low and sub-zero temperatures were investigated on *Genipa americana* seeds. The optimum temperature for germination of fresh seeds was found to be 30°C. The critical moisture content (seed viability reduced to 50%) for the seeds was between 9 and 6%. Germination capacity was maintained for 12 months during storage at 5, 10 and 15°C, when seeds were at *ca.* 11% moisture content. A drastic reduction of seed viability occurred after -20°C exposure. These results suggest that *Genipa americana* seeds have intermediate behaviour.

### **Introduction**

*Genipa americana* L. (local name: jenipapo) is a member of the Rubiaceae family that occurs in humid Brazilian ecosystems. The wood of the species is used for many purposes. The Indians use the blue juice made from immature fruits to dye their skin. The mature fruits are edible and used to prepare ice cream, pudding, juice, wine and liquor (Villachica *et al.* 1996). The seed has a flat and irregular, sometime rectangular, shape. The seed coat is thin and yellowish-brown and the endosperm is yellowish-white.

Classifying these seeds into the correct storage behaviour and establishing appropriate conditions for germplasm conservation are important for the long-term conservation of *G. americana*. The seeds have been found to have a short lifespan, however, there are conflicting reports of actual seed storage category with suggestions of both recalcitrant and intermediate seed storage behaviour (Lorenzi 1992; Carvalho and Nascimento 2000). In this study, the responses to germination temperature, desiccation and storage conditions were investigated.

## **Materials and methods**

### **Seed collection and extraction**

Fruits were collected in 1998 from three trees 1 km apart, in the savannah vegetation at Mangabeira farm (access road BR 080) in the Mato Seco region, state of Goiás. The seeds were extracted by hand. After removing the fleshy pulp by rubbing the fruits in a sieve, the seeds were washed in tap water. The same day, moisture content determinations and desiccation trials were initiated.

Fruit and seed weights were determined for 100 individual fruits and their seeds. Initial moisture content was determined for 100 individual seeds and for five replicates of each of five whole seeds, 20 isolated embryonic axes, and 20 isolated endosperms plus seed coats.

### **Effect of temperature on seed germination**

Four replicates of 25 fresh (nondried) seeds were germinated on moistened paper towel, at a range of constant temperatures between 5 and 40°C with 12 h light per day. Samples of seeds were dried at room temperature (25±2°C) by mixing with equal amounts of silica gel. Control samples were placed in similar containers with vermiculite instead of silica gel. The desiccation periods were from 0 up to 72 h. After each desiccation period, MC was determined on five replicates of five whole seeds, and germination tests were carried out with four replicates of 25 seeds each, at 30°C with 12 h light per day.

### **Seed desiccation and response to -20°C**

Further samples of ≥225 seeds each were dried at room temperature with silica gel, as before, for between 24 and 72 h. After desiccation, moisture content (MC) was determined using five replicates of five whole seeds each and germination tests were carried out with four replicates of 25 seeds, incubating them at 30°C with 12 h light per day. The remaining 100 dried seeds were placed at -20°C for 24 h before sowing for germination at 30°C, as described above.

## Storage trials

Seeds treated with fungicide were dried to four different moisture contents. Sub-samples were then mixed with vermiculite, sealed in impermeable bags, and stored at 5, 10 and 15°C for 2, 4, 6, 8, 10 and 12 months. Seeds were regularly taken to test their germination capacity as described above.

## Results

Mean fruit and seed weights were  $175.5 \pm 53.2$  g and  $0.09 \pm 0.01$  g, respectively. The initial MC of whole seeds was ca. 44–46% fresh weight (Table 1). For seed tissues, the initial MC was higher for embryonic axes (76%) compared with seed coats and endosperms (37%; Table 1).

**Table 1.** Initial moisture contents of seeds and seed tissues

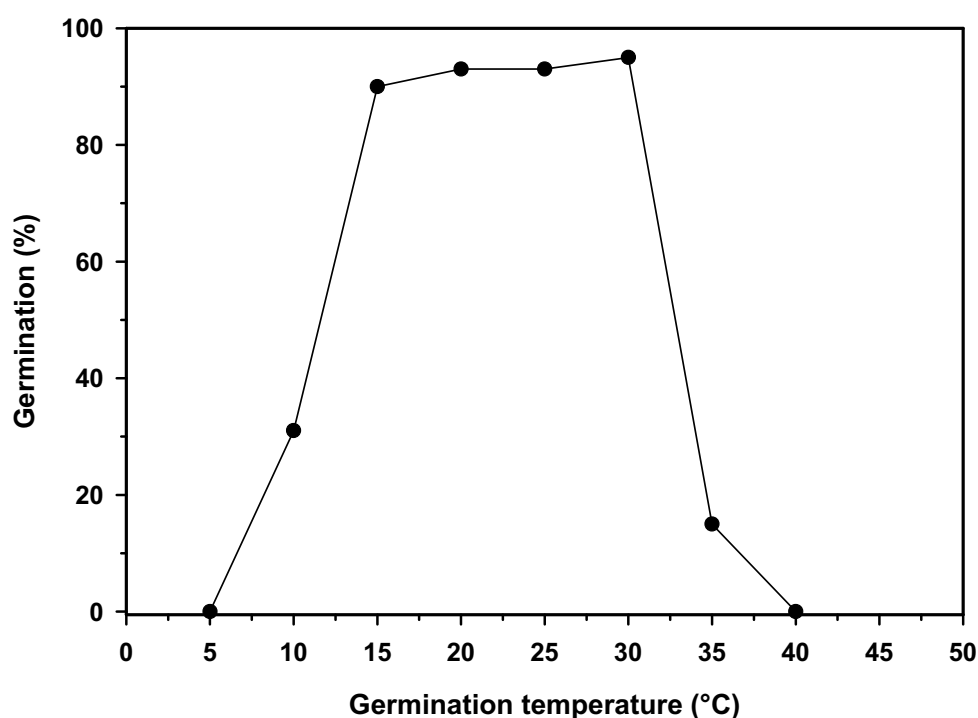
Material	MC $\pm$ s.d. (%)
Mean of 100 individual seeds	43.8 $\pm$ 2.60
Mean of five replicates of five whole seeds	46.0 $\pm$ 5.31
Mean of five replicates of 20 embryonic axes	76.5 $\pm$ 0.69
Mean of five replicates of 20 endosperms plus seed coat	38.0 $\pm$ 0.69

## Effect of temperature on seed germination

Maximum germination was achieved at temperatures between 15 and 30°C, while no seeds germinated at 5 and 40°C (Fig. 1). At 10°C, radicles protruded from 97% of the seeds, but only 31% developed into seedlings; at 15, 20, 25 and 30°C, normal seedlings developed within 84, 46, 26 and 22 days, respectively. Radicles of germinated seeds at 35°C were necrosed.

## Desiccation trials

High germination percentages were maintained for seeds dried to moisture contents between 47 and 19% (Table 2). A slight decrease in viability (to 78%) was observed for seeds dried to 9% MC, whereas viability was reduced to 29–43% germination in seeds dried to approximately 7% MC.



**Figure 1.** Effect of temperature on the germination of *G. americana* seeds. Each data point represents mean of four replicates of 25 seeds.

**Table 2.** Effect of desiccation (MC%) on seed germination capacity (Germ.%)

Desiccation (h)	Controls (vermiculite)		Desiccated seeds (silica gel)	
	MC±s.d. (%)	Germ. (%)	MC±s.d. (%)	Germ. (%)
0	56.48±1.46	96	—	—
3	51.48±1.12	99	47.43±0.90	91
5	53.88± 0.65	97	45.41±1.29	87
8	53.33±2.87	97	43.95±2.16	93
12	54.01±0.24	94	36.79±1.75	97
16	53.12±1.08	100	34.84±1.79	97
20	52.61±0.79	99	25.20±2.29	96
24	50.84±1.70	99	19.23±2.51	92
36	54.49±1.07	99	9.34±1.03	78
48	53.12 ±1.39	96	6.88±0.24	43
72	48.31±0.45	100	6.72±0.25	29

### Effect of desiccation and exposure to $-20^{\circ}\text{C}$ on seed viability

Low germination percentages (between 0 and 15%) were obtained for seeds at all moisture contents placed at  $-20^{\circ}\text{C}$  for 24 h (Table 3). The best result of 15% germination was observed for seeds dried to 8.6% MC, while viability was no more than 3–4% at moisture contents above or below this level.

**Table 3.** Effect of desiccation and exposure to  $-20^{\circ}\text{C}$  for 24 h on seed viability

Desiccation (h)	MC (%)	Germination (%)	
		Before $-20^{\circ}\text{C}$	After 24 h at $-20^{\circ}\text{C}$
0	51.5 $\pm$ 1.10	99	—
24	13.2 $\pm$ 2.20	66	3
28	8.6 $\pm$ 0.23	61	15
48	7.1 $\pm$ 0.24	41	4
52	6.5 $\pm$ 0.65	31	1
72	6.3 $\pm$ 0.09	33	0

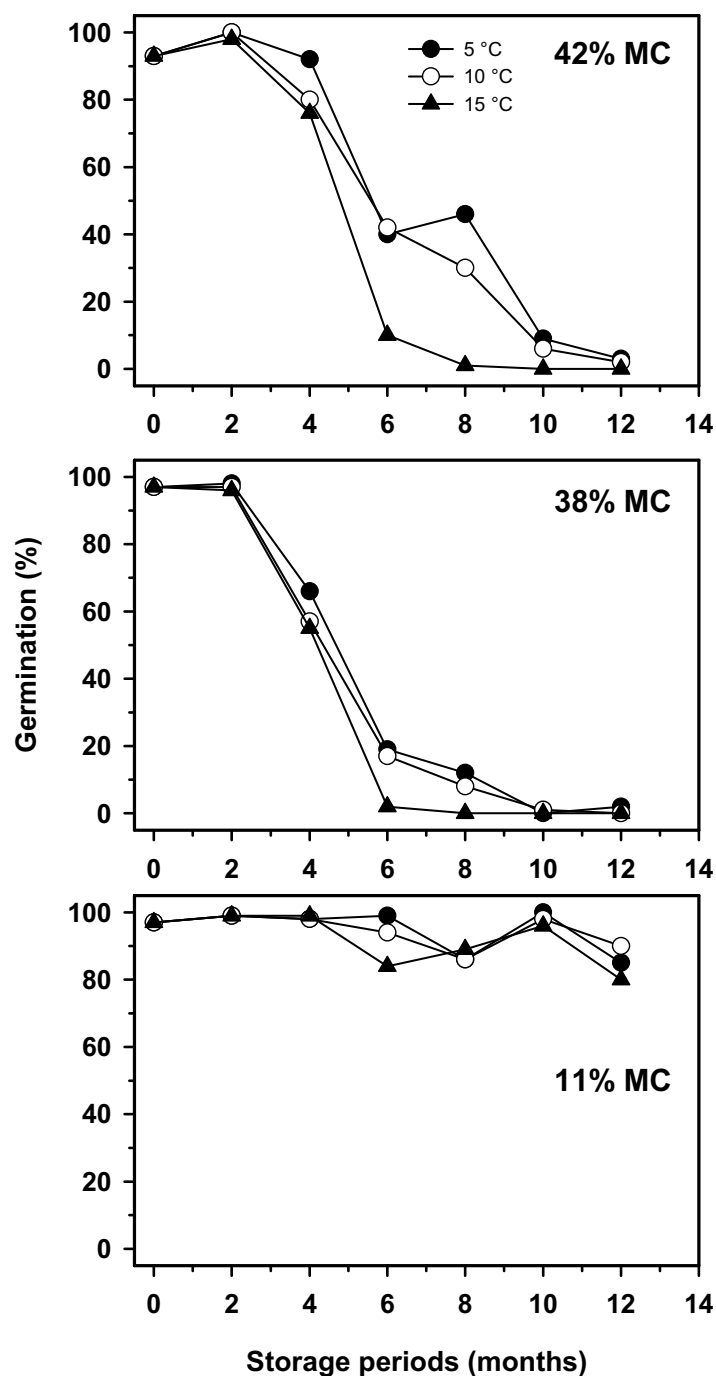
### Storage trials

Seed viability decreased during storage at 5, 10, and  $15^{\circ}\text{C}$  (Fig. 2). Rate of loss of viability appeared to be fastest for seeds at 38% MC. However, for seeds at both 42% and 38% MC there was no germination after 12 months storage at 5, 10 or  $15^{\circ}\text{C}$ . High levels of germination (>80%) were maintained in seeds dried to 11% MC and stored for 12 months at 5, 10 or  $15^{\circ}\text{C}$ .

### Discussion

Drying seeds of *G. americana* resulted in a decrease in germination percentage, the critical MC being somewhere between 9 and 6% fresh weight (Tables 2 and 3). Although the seeds showed sensitivity to  $-20^{\circ}\text{C}$  (Table 3), germinability was maintained during 12 months storage at 5, 10 and  $15^{\circ}\text{C}$ , when seeds were stored with ca. 11% moisture content. These results show that *G. americana* seeds are not recalcitrant and may have intermediate storage behaviour. Unfortunately, the survival of seedlings in the greenhouse was compromised by *Fusarium oxysporum* contamination.

A preliminary test showed that regeneration of *G. americana* embryonic axes after desiccation and exposure at  $-196^{\circ}\text{C}$  was unaffected by bacteria contamination. Therefore, it is suggested to develop a cryopreservation protocol for embryonic axes of this species.



**Figure 2.** Germination response of *G. americana* seeds dried to 42, 38 and 11% MC and stored at 5, 10 and 15°C for 12 months.

## References

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