Germination of stored *Belamcanda chinensis* (L.) DC seeds [1]

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ABSTRACT

The leopard lily (*Belamcanda chinensis*) is an exotic ornamental Iridaceae in Brazil and cultivated as a medicinal plant in its countries of origin (China and Japan). It is propagated by rhizomes and seeds; however, the viability of these seeds after storage remains unknown. The aim of this study was to evaluate the germinability of stored *B. chinensis* seeds. Seeds harvested between March and April 2010, 2011, 2012 and 2013 were separated by year and stored in paper bags in a room without relative humidity and temperature control. Seeds from these four years were analyzed for moisture content, 100-seed weight and germination index. The freshly harvested seeds (2013) and those stored for one year (2012) exhibited 72% and 41% germination, respectively, and 80% and 47% primary root emission at the end of 20 weeks. However, seeds stored for two and three years (2010 and 2011) did not germinate. *B. chinensis* seeds remained viable for up to one year of storage.
Keywords: Iridaceae, conservation, normal seedlings, seed vigor.

RESUMO

Germinação de sementes armazenadas de *Belamcanda chinensis* (L.) DC

A flor-leopardo (*Belamcanda chinensis*) é uma iridácea ornamental exótica no Brasil e cultivada, em seus países de origem (China e Japão), como planta medicinal. A sua multiplicação se dá por rizomas e sementes. Contudo, não há relatos sobre a viabilidade das sementes desta espécie após o armazenamento. Assim, objetivou-se com este trabalho avaliar a armazenabilidade de sementes de *B. chinensis*. Para tanto, sementes colhidas entre março e abril de 2010, 2011, 2012 e 2013 foram acondicionadas em sacos de papel e armazenadas, separadamente por ano, em sala sem controle de umidade relativa e temperatura. Em sementes desses quatro anos foram determinados o teor de água, a massa de 100 sementes e o índice de germinação. As sementes recém-colhidas (2013) e as armazenadas por um ano (2012) apresentaram, respectivamente, 72% e 41% de germinação e 80% e 47% de emissão de raiz primária ao final de 20 semanas. No entanto, as sementes com dois e três anos de armazenamento (2010 e 2011) não germinaram. Portanto, as sementes de *B. chinensis* apresentam viabilidade por até um ano de armazenamento.
Palavras-chave: Iridaceae, conservação, plântulas normais, vigor de sementes.

1. INTRODUCTION

The ability of seeds to germinate can change during storage, since they start to deteriorate after harvest and ultimately lose their viability. This has ecological and agronomical implications for understanding the mechanisms underlying loss of vigor during seed storage (NGUYEN et al., 2015).

Knowledge of the time period during which seeds maintain their ability to germinate is important for any cultivated species. As such, it is necessary to know the best seed conservation conditions, moisture content being one of the critical factors in identifying storage performance, since seeds that cannot withstand drying may not survive storage, precluding their use in planting (TOMBOLATO et al., 2009; SILVA et al., 2015; SOUZA et al., 2016).

Among the factors that most affect the storage environment and seed conservation are temperature and relative humidity (BASS, 1980; DOIJODE, 2001; SCHORN et al., 2010; UMARANI et al., 2015; VENIAL et al., 2017). Sword lily (*Gladiolus hortulanus* L., family Iridacea) seeds exhibit orthodox storage behavior, remaining viable for one year under ambient laboratory conditions, although at a lower germination percentage, with optimal storage conditions of 14 °C and 26% relative humidity (CARPENTER et al., 1991). On the other hand, information on the best storage conditions for the species *Belamcanda chinensis* nonexistent.

B. chinensis, of Asian origin, belongs to the family Iridaceae and is widely used in Brazil as an ornamental plant (LORENZI and SOUZA, 2001). In China and Japan,

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it is used as a medicinal herb (ZHANG et al., 2016; XIN et al., 2015). The leopard lily blooms in January or February and its fruits ripen between April and June. It has orange flowers with reddish spots and each flower is approximately four cm in diameter, has six petals, three stamens and one tripartite stigma (XIN et al., 2015), characteristics with ornamental potential.

Owing to the difficult germination and lack of knowledge on the storage capacity of *B. chinensis* seeds (SILVA et al., 2017), studies investigating the storage capacity of these seeds are needed to ensure their availability in seed conservation programs. Given these aspects reported in the literature, the aim of this study was to explore the storage feasibility of *B. chinensis* seeds.

2. MATERIAL AND METHODS

B. chinensis seeds collected from dehiscent fruits in March and April 2010, 2011, 2012 and 2013 were separated by year in single-layer Kraft paper bags, and stored in a room with no temperature or relative humidity control. Temperature and relative humidity variations for the region were obtained from the following sources: http://clima.icea.gov.br/ and https://www.agritempo.gov.br/ (Table 1).

Table 1. Mean data of the minimum and maximum temperature and relative humidity of Botucatu, São Paulo State, Brazil - Years 2010 to 2013

	Temperature °C			
	2010	2011	2012	2013
Minimum	6.0	1.0	6.0	4.0
Maximum	25.7	36.0	36.0	38.0
	Relative humidity (%)			
Minimum	56.8	52.9	48.3	62.5
Maximum	87.6	86.1	71.1	73.2

Source: http://clima.icea.gov.br/; https://www.agritempo.gov.br/

The predominant climate in the municipality of Botucatu, São Paulo (SP) State, where the study was conducted, is humid subtropical (Cfa), under the Köppen climate classification, with hot humid summers and cold dry winters. Rainfall is concentrated between November and April, with an annual average of 1324 mm.

In May 2013, the seeds from these four growing seasons were assessed for moisture content (%), 100-seed weight (g), primary root emergence (%), germination (%) (normal seedlings), primary root emergence and germination speed (number/week), and average primary root emergence and germination time (weeks).

Seed moisture content was assessed using the oven method at 105 ± 3 °C for 24 h, with 2 replications (BRASIL, 2009) involving 25 seeds each. The 100-seed weight was determined in four repetitions with 100 seeds, using an electronic balance accurate to 0.0001 g, and moisture content was corrected to 9.7% for all the treatments.

The germination test was conducted with four repetitions of 50 seeds, germinated on two sheets of paper towel and covered with another sheet, all previously moistened with distilled water, at a ratio of 2.5 times the substrate dry weight. The sheets were rolled up and placed in a sealed plastic bag, which was kept in a seed germinator at temperatures alternating between 20-30 °C, with eight hours of light at the highest temperature (SILVA et al., 2017).

Primary root emergence of 2 mm and normal seedlings (BRASIL, 2009) were counted weekly for 20 weeks. These data were used to calculate the percentages, speed indices and average primary root emergence and germination times. Speed indices were calculated based on Maguire (1962) and average times according to Santana and Ranal (2000), which considered the time period between seeding and counting dates in weeks.

The data underwent statistical analysis, in a completely randomized design, and were transformed into y = arcsen $\sqrt{x/100}\sqrt{x/100}$ or $y = \sqrt{x+0.5}$ $y = \sqrt{x+0.5}$ when necessary (GOMES, 2000). Tukey's test at 5% probability was used to compare the averages.

3. RESULTS AND DISCUSSION

The moisture content was around 3% higher in freshly harvested *B. chinensis* seeds (2013) when compared to previous years, although all collections occurred in the dry season, with dehiscent fruits and seeds exposed to sunlight (Table 2). Seeds harvested in 2010, 2011 and 2012 were stored in porous paper bags and exposed to oscillations in moisture and room temperature for longer, which may have favored moisture loss during storage, resulting in lower content than in freshly harvested seeds. As such, the loss of moisture in the stored seeds suggests that they were in hygroscopic balance with the relative air humidity (SANTOS et al., 2005).

Table 2. Mean untransformed data of water content (TA), mass of 100 seeds (M100), primary root emission (ER), germination (G), abnormal seedlings (PA), and dead seeds (SM) of *B. chinensis* stored by three (2010), two (2011), one year (2012) and freshly harvested (2013).

Treatments (years)	TA (%)	M100 (g)	ER (%)	G (%)	PA (%)	SM (%)
2010	9.3^{b}	3.236^{bc}	0^{c}	0^{c}	0^{b}	100^{a}
2011	9.7^{b}	2.995^{c}	0^{c}	0^{c}	0^{b}	100^{a}
2012	9.9^{b}	3.348^{ab}	47^{b}	41^{b}	6^{a}	53^{b}
2013	12.2^{a}	3.580^{a}	80^{a}	72^{a}	8^{a}	20^{c}
CV (%)	2.57	4.65	6.31	8.9	23.56	3.52

* Means followed by equal letters in the same column do not differ by Tukey test at 5% probability.

In seeds harvested in 2013, the 100-seed weight was greater (0.58 g) than in 2010 and 2011 (Table 2), indicating that the environmental conditions were more favorable in that year, resulting in better seed development and weight (Table 2). These data corroborate those reported by Carvalho and Nakagawa (2000) and Toledo et al. (2009), since, according to these authors, heavier or denser seeds are better nourished during their development and tend to exhibit enhanced physiological quality.

The germination process of *B. chinensis* was slow, with primary root emergence in the third week and normal seedling formation from the fifth week onwards (Table 2). Thus, germination in *B. chinensis* is slower than for seeds of other ornamental species of the family Iridaceae, since the final count for *Fresia refracta* is recommended at 35 days, for *Gladiolus spp.* at 16 days and for *Iris kaempferi* at 18 days (BRASIL, 2009). Assessment of *B. chinensis* germination in the present study occurred in the twentieth week after the start of the test, corroborating the results of Silva et al. (2017). Similar to these three species for which procedures to overcome dormancy are recommended (BRASIL, 2009), *B. chinensis* also seems to display physiological dormancy. Thus, more studies are needed to establish germination testing procedures to overcome this property (SILVA et al., 2017).

With respect to germination percentage of seeds submitted to the four treatments (Table 2), those freshly harvested individuals (2013) obtained statistically higher results than in the other years, with a 73% germination rate, followed by seeds stored for one year (2012), with 41% for both primary root emergence and germination (normal seedlings). These results were also reported for *Caesalpinia echinata* Lam. (BARBEDO et al., 2002) and *Moringa oleifera* Lam. (BEZERRA et al., 2004), which showed less germinative capacity when freshly harvested seeds were stored in a natural environment. Thus, seeds deteriorate naturally over time and lose vigor due to biochemical and physiological damage (DONADON et al., 2015; FELIX et al., 2017). Seeds stored for two (2011) and three years (2010) did not produce normal seedlings or primary roots, all of them being dead (Table 2). Therefore, storing *B. chinensis* seeds did not favor conservation of physiological seed quality for more than two years. Oscillations in relative air humidity and ambient temperature may have negatively affected the physiological quality of *B. chinensis* during storage.

The presence of more dead seeds among those stored for one year (2012) compared to seeds tested in the harvest year (2013) explains the different germination between them, because the occurrence of abnormal seedlings was similar (Table 2). The relatively high percentage of dead freshly harvested seeds (2013) may be related to the germination test conditions, which were not those recommended by Silva et al. (2017), causing them to deteriorate during the test. However, for the variable dead seeds, few were visually infested or contained microorganisms, even those stored for longer than two years (100% mortality).

Similarly to the percentage results, the primary root emergence and germination speeds (Table 3) were lower, with a difference of 1.05 for the former and 1.07 for the latter in seeds stored for one year (2012) compared to those freshly harvested (2013), indicating better quality (vigor) in the recently harvested seeds. However, it is important to underscore that even under natural storage conditions with no temperature or relative humidity control, *B. chinensis* seeds continued to germinate for one year, albeit with decreased physiological potential. A similar occurrence was observed in the sword lily (CARPENTER et al., 1991), also from the family Iridaceae, as well as in *Acacia polyphylla* DC (ARAÚJO NETO et al., 2005), *Tabebuia impetiginosa* (Mart.) (BORBA FILHO and PEREZ, 2009) and apple of Sodom (OLIVEIRA-BENTO et al., 2015), whose physiological quality is more negatively affected in a natural environment, where temperature and relative air humidity are not controlled, when compared to other storage environments.

Table 3. Untransformed average data of primary root emission rate index (IVER), germination rate index (IVG), mean time of primary root emission (T-ER) and medium germination time (T-G) of *B. chinensis* seeds stored for three (2010), two (2011), one year (2012) and freshly harvested (2013).

Treatments (years)	IVER (N°/Weeks)	IVG (N°/ Weeks)	T-ER (Weeks)	T - G (Weeks)
2010	0[c]	0[c]	0[c]	0[c]
2011	0[c]	0[c]	0[c]	0[c]
2012	3.87[b]	2.62[b]	6.56[b]	8.17[b]
2013	4.92[a]	3.69[a]	9.20[a]	10.46[a]
CV (%)	4.83	5.15	3.50	3.64

* Means followed by equal letters in the same column do not differ by Tukey test at 5% probability.

Although percentages and velocity indices were higher in freshly harvested seeds (2013), the average primary root emergence and germination times (Table 3) were lower in those stored for one year (2012), showing greater germination speed than in new seeds. This apparent contradiction no longer exists if seed dormancy is considered, with a greater presence and intensity in freshly harvested individuals compared to their stored counterparts (DOIJODE, 2001; BESSA et al., 2015).

The difference in germination was demonstrated by the average primary root emergence and germination times, which showed the effect of the greater dormancy in new seeds, with higher average times (Table 3). The differences in physiological quality, reflected in the germination, 100-seed weight and velocity indices of *B. chinensis* seeds, stored or not, may be attributed to storage conditions (SMANIOTTO et al., 2014), since seed characteristics (100-seed weight and moisture content) were not very different between the harvest years (Table 2), which could have interfered in the physiological assessment results.

4. CONCLUSIONS

B. chinensis seeds stored for one year in a natural environment was viable, but exhibited lower physiological quality than freshly harvested seeds, which caused a decline in germination.

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AUTHORS CONTRIBUTIONS

G.H.S.: discussion and data interpretation, writing and revision of the manuscript. **L.S.B., C.A.T., R.F.R.**: material collection, setting up tests, data collection and tabulation. **J.N.**: data analysis and interpretation, critical revision and final approval of the manuscript.

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