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Physalis peruviana seed storage

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Key words:

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ABSTRACT

Physalis peruviana belongs to Solanaceae family and has a high nutritional and nutraceutical potential. The production is intended for fruit consumption and the propagation is mainly by seeds. This study aimed to evaluate the influence of priming on the kinetics of germination of *P. peruviana* seeds stored at different temperatures. The seeds were stored at 5 and 25 °C in a chamber saturated with zinc chloride solution and in liquid nitrogen (-196 °C). Every 4 months, the seeds were removed from storage for evaluation of germination and moisture content in the laboratory and emergence and development of seedlings in greenhouse. During the last evaluation at 16 months, the seeds under the same conditions were subjected to salt stress. The moisture content varied during the storage period, but was always higher for seeds kept at -196 °C. These seeds kept high germination percentage in water until 16 months, regardless of the tested temperature; however, in salt solution the germination percentage was significantly reduced.

Palavras-chave:

osmocondicionamento
criopreservação
solução salina
viabilidade

Armazenamento de sementes de *Physalis peruviana* L.

RESUMO

Physalis peruviana pertence à família Solanaceae; possui alto potencial alimentício e nutracêutico. A produção ocorre para consumo dos frutos e a propagação se dá principalmente via sementes. Objetivou-se, no presente trabalho, avaliar a influência do osmocondicionamento na cinética da germinação de sementes de *P. peruviana* armazenadas em diferentes temperaturas. As sementes foram armazenadas em temperaturas de 5 e 25 °C em câmara saturada com solução de cloreto de zinco e em nitrogênio líquido (-196 °C). A cada 4 meses as sementes foram retiradas do armazenamento para avaliação da germinabilidade e do teor de umidade em laboratório, emergência e desenvolvimento de plântulas em viveiro. Durante a última avaliação, aos 16 meses, as sementes nas mesmas condições foram submetidas ao estresse salino. O teor de umidade variou durante o período de armazenamento sendo sempre superior para as sementes mantidas a -196 °C as quais mantiveram alta porcentagem de germinação em água até os 16 meses, independente da temperatura testada; entretanto em solução salina a porcentagem de germinação foi significativamente reduzida.



INTRODUCTION

Some botanical families, such as Solanaceae, have been well documented with respect to the various uses of their species. These species include plants with medicinal use due to the presence, in stems and leaves, of secosteroids, a group of molecules generically known as physalins (Tomassini et al., 2000; Giorgetti & Negri, 2011).

Besides the proven therapeutic use of *Physalis angulata*, other species are widely used as food, such as *P. peruviana* L. and *P. ixocarpa* B; others are used as ornamental and some are considered as toxic (Rufato et al., 2008; Vargas-Ponce et al., 2011).

In Brazil, *P. peruviana* fruits are considered as exotic, with high market value, and can be incorporated to the cultivation of small fruits (Lima et al., 2009). Combined with the economic appeal, it is considered as a good source of natural antioxidant compounds (Rockenbach et al., 2008), besides other components, such as vitamins A, B, C, E and K1, phytosterols, essential minerals and secosteroids (Puente et al., 2011).

P. peruviana can be propagated asexually with the use of stem cuttings or in vitro cultivation; however, the main form of multiplication is through sexual means, using seeds with high germination percentage (up to 90%) (Rufato et al., 2008). Since the seed is a natural propagation unit, ex-situ conservation is the most used form, because it occupies small space and requires only periodic monitoring, thus constituting an efficient method to preserve the germplasm (Santos, 2000; Slageren, 2003). This is a process that requires knowledge on plant cultivation conditions, which will reflect in the quality of the obtained seeds.

Seed aging can be delayed if the seeds are stored under appropriate conditions, which restrict the advance of the processes of natural deterioration. Among the biotic and abiotic processes that influence seed quality, there are temperature, relative air humidity and insects and fungi (Barua et al., 2009). High temperatures and relative air humidity increase cell respiration, while low temperatures delay metabolic processes and inhibit infestation (Barua et al., 2009).

In this context, a method that has been frequently used for seed conservation is cryopreservation. This technique is characterized by the maintenance of plant segments (seeds or other plant parts) in liquid nitrogen (-196 °C) or vapor nitrogen (-150 °C) (Kantha, 1985). Under these conditions, the metabolic processes are maintained in a latent state, minimizing deterioration and allowing long-term conservation (Stanwood, 1985).

For a successful storage, the collected seeds must have high quality and vigor. However, collecting seeds with high vigor is not always possible. Thus, a few techniques may help and increment the kinetics of the germination of seeds of various species. The priming consists of a pre-treatment in which seeds are immersed in osmotic solution under controlled time and temperature, in order to restrict the amount of absorbed water (Anwar et al., 1978; Wien, 1997). In large plant productions, techniques like priming are desirable, for increasing germination, emergence speed and percentage at the field and also improving the performance under stress conditions (Gomes et al., 2012).

This study aimed to evaluate the influence of priming on the kinetics of germination of *P. peruviana* seeds stored at different temperatures.

MATERIAL AND METHODS

Ripe fruits of *P. peruviana* were collected from plants maintained at the Horto Florestal Experimental Unit of the State University of Feira de Santana-BA (UEFS) in September 2012. After collection, the seeds were removed from the fruits, washed and placed in incubators saturated with calcium chloride solution at 20 °C for 24 h, until equilibrium of moisture. From the obtained lot, one third of seeds was osmoconditioned in aerated solution of polyethylene glycol (PEG 6000), according to the methodology defined by Villela et al. (1991), at osmotic potential of -0.8 MPa for 10 days, in germination chamber at 25 °C (previously established methodology). On the 10th day, the seeds were removed from the PEG solution, washed and dried as previously described. Samples of primed and non-primed seeds were separated, stored in refrigerator (5 ± 3 °C), in germination chamber (25 ± 3 °C) and in liquid nitrogen (-196 °C). Primed and non-primed seeds, before being stored, were considered as a control treatment. In refrigerator and in germination chamber, the seeds were maintained in containers with solution saturated by zinc chloride (±4% of relative humidity). In the cryopreservation, the seeds were previously desiccated in a container with silica gel for 60 min and then stored; seeds were slowly unfrozen, at room temperature, for 3 h.

The storage occurred for 16 months and, in each interval of 4 months, seed samples were collected from each environment for the determination of the water content (Brasil, 2009) and the tests of germination, at the laboratory, and emergence, at the field. For the germination test, seeds were arranged on Petri dishes (6 cm) with germitest paper containing 3 mL of distilled water. The tests were performed in germination chamber at 25 °C with photoperiod of 12 h and four replicates of 25 seeds for each treatment. The evaluations occurred on a daily basis and the seeds with 1 mm of root were considered as germinated.

For the emergence test, seeds of the same treatments described above were planted in polystyrene trays containing the commercial substrate Plantmax and arranged in a controlled environment (40% luminosity and irrigation). The emergence was evaluated at 7, 14 and 21 days after sowing (DAS), besides plantlet normality rate (%) (Brasil, 2009).

Primed and non-primed seeds, stored for 16 months in refrigerator (5 ± 3 °C), in germination chamber (25 ± 3 °C) and in liquid nitrogen (-196 °C) were subjected to saline solution (sodium chloride with electrical conductivity of 4 dS m⁻¹). The following parameters were evaluated: seed water content, germination percentage (G%), germination mean time (T_m) and germination speed index (GSI).

The treatments were arranged in a completely randomized design, in split-plots along the time, and four replicates. Storing environments were considered as plots and the combination

of pre-treatment factors (seed priming) and storage time were considered as subplots. The obtained data were tested for normality and homogeneity, through the tests of Shapiro-Wilk and Bartlett at 0.05 probability level, and were transformed to arcsine when necessary. The data that did not show normality and homogeneity even after transformation were not presented. The control treatments (T0) were compared with the others by the Dunnett test at 0.05 probability level.

In the salinity test, the treatments were arranged in a completely randomized design, in a 2 x 2 x 3 factorial scheme, with 2 types of seeds (with and without priming), 2 conditions (water and saline solution) and 3 storage temperatures (5, 25 and -196 °C).

RESULTS AND DISCUSSION

The water content of *P. peruviana* seeds varied during the storage period, but did not exceed 11%, regardless of the adopted treatment. Initially, primed and non-primed seeds showed contents of 5.9 and 4.1%, respectively; at 16 months, these contents increased to 7.0 and 7.4% at temperature of 25 °C, 8.4 and 8.6% at temperature of 5 °C and to 10.7 and 10.1% for cryopreserved seeds, after unfreezing. Cryopreserved seeds showed higher water contents compared with those maintained in chamber with zinc chloride, regardless of the temperature and storage time. Such increase in water content can be attributed to the freezing-unfreezing process, which may promote the formation of ice crystals in the tissues of seeds maintained in liquid nitrogen (Santos, 2000). The use of zinc chloride in saturation chamber, at temperatures of 25 and 5 °C, was efficient to maintain water contents low and closer to those observed initially.

The most relevant factor for seed conservation is its water content (Labbé, 2003). Water contents above 13% are not desirable for storage, because, as hygroscopic structures, the seeds are able to perform exchanges with the environment and can lose or get moisture (Labbé, 2003). Maintaining low water contents for orthodox seeds may be one of the reasons why the germination percentage and vigor of seeds remained high along the all evaluation period (Table 1). The influence of the storing environment was not significant in the performed analyses.

There was significant difference at 8 months, when non-primed seeds showed higher germination percentage in shorter time; however, at 16 months, the behavior inverted and there

Table 1. Germination percentage (G), germination mean time (Tm) and germination speed index (GSI) of types of seeds (TS) of primed (P) and non-primed (NP) *P. peruviana* seeds at different storage periods

Variables	TS	Storage time (months)			
		4	8	12	16
G (%)	P	98.00 A	96.00 B	98.66 A	98.33 A
	NP	96.66 A	99.00 A	100.00 A	97.66 A
Tm (days)	P	6.96 A	8.28 B	7.28 A	5.96 A
	NP	7.07 A	6.35 A	7.32 A	7.35 B
GSI	P	3.84 A	3.44 B	3.70 A	4.32 A
	NP	3.57 A	4.20 A	3.53 A	3.50 A

Means followed by the same letter in the column for each evaluated variable do not differ by Tukey test at 0.05 probability level

was an increase in germination and a significant reduction in the germination mean time of primed seeds, compared with the non-primed ones. These data corroborate those of Souza et al. (2014b), who observed that primed seeds *P. angulata* seeds before and after storage at 15, 21 and 24 months showed higher germination percentage in comparison to the non-primed ones. Studies have shown that priming is a useful technique, especially for lots of seeds with low vigor, from economically important species (Soeda et al., 2005; Flors et al., 2007; Varier et al., 2010).

According to Varier et al. (2010), the priming technique increases the longevity of low vigor seeds and decreases it in high-vigor seeds, because, in the former, it allows metabolic repairs before germination, preventing later deterioration. Thus, recently collected and primed seeds may have their performance harmed in relation to non-primed ones. At 8 months, primed seeds spent significantly longer time to germinate at all storage temperatures (Table 2), in comparison to recently collected and primed seeds and non-primed seeds.

According to Delouche (2002), there are other forms of detecting seed deterioration before the occurrence of significant losses of germination. Thus, for the evaluation of vigor of stored seeds, the percentages of emergence and number of normal seedlings in the greenhouse were also observed, which were significant for the interaction of temperature versus storage time (Table 3).

There are several effects of seed deterioration as damages in the membrane, decrease in germination speed, growth and

Table 2. Mean time of germination of types of seeds (TS) of primed (P) and non-primed (NP) *P. peruviana* seeds at different temperatures and storage periods, compared with the control (initial time)

Storage temperature (°C)	TS	Mean time (days)			
		Storage periods (months)			
		4	8	12	16
25	P	7.07	8.65**	6.77	6.51
	NP	7.76	6.85	7.39	6.72
5	P	6.53	7.95*	7.30	5.46
	NP	6.68	6.01	7.35	7.72
-196	P	7.30	8.14**	7.78	5.91
	NP	6.77	6.21	7.23	7.62
O (Initial time)		6.20*			
NO (Initial time)		6.50#			

Means followed by * do not differ from the osmoconditioned control and means followed by # do not differ from the non-osmoconditioned control at the initial time by the Dunnett test at 0.05 probability level

Table 3. Percentages of emergence at 14 days and normal plantlets at 21 days for primed and non-primed *P. peruviana* seeds at different storage periods

Variables	Storage temperature (°C)	Storage period (months)			
		4	8	12	16
Emergence (%)	25	87.00 A	77.00 B	82.50 A	81.50 A
	5	79.00 A	89.00 A	83.50 A	67.50 B
	-196	79.50 A	86.12 A	79.00 A	81.50 A
Normal plantlets (%)	25	85.33 A	70.65 B	92.35 A	84.75 A
	5	82.67 A	89.46 A	88.94 A	81.45 A
	-196	78.53 A	83.61 A	86.87 A	82.37 A

Means followed by the same letter in the column do not differ by Tukey test at 0.05 probability level

development of seedlings, decrease in resistance or tolerance to environmental and emergence stresses, increase in the number of abnormal seedlings until culminating in the total loss of germination capacity (Delouche, 2002).

The results showed high emergence percentage at 14 days in all the evaluated storage periods, getting maximum of 89%, with reduction at 16 months, 67.5% (Table 3). The reduction in emergence percentage did not affect the number of abnormal seedlings, which remained very similar during the evaluations.

At 8 months, it was possible to observe a decrease in emergence percentage and normal seedlings for the seeds stored at temperature of 25 °C. These data agree with those of Torres et al. (2005), who describe that high temperatures influence the speed of biochemical processes and also seed water content, affecting its metabolism, culminating in its deterioration. For *P. angulata*, the germination rate of seeds primed seeds and stored at room temperature (25 °C) was high (above 90%) until 24 months (Souza et al., 2014b). Seeds of tomato (*Lycopersicon lycopersicum* L.) priming for 7 days and stored at 10 °C remained viable during 12 months, but at 30 °C the viability was reduced (Alvarado & Bradford, 1988).

The analysis of variance of the salt stress experiment showed highly significant isolated effect for the treatment with stress (Table 4). Among the abiotic stresses, salinity stands out

Table 4. Summary of the analysis of variance for the studied parameters in two types of seeds of *P. peruviana* stored at three different temperatures for 16 months and subjected to saline stress

Source of variation	DF	Mean square		
		G (%)	Tm (days)	GSI
Type of seed (TS)	2	0.12 ^{ns}	0.46*	0.19 ^{ns}
Stress (S)	1	108.19**	12.34**	10.19**
Storage period (SP)	2	4.01 ^{ns}	0.22 ^{ns}	0.18 ^{ns}
TS * S	2	0.21 ^{ns}	0.07 ^{ns}	0.09 ^{ns}
TS * SP	4	0.19 ^{ns}	0.12 ^{ns}	0.01 ^{ns}
S * SP	2	3.25 ^{ns}	0.18 ^{ns}	0.14 ^{ns}
TS * S * SP	4	0.19 ^{ns}	0.09 ^{ns}	0.02 ^{ns}
Residue	54	1.76	0.08	0.06
CV (%)		15.74	9.06	16.02
Mean		70.9	9.49	2.31

DF – Degrees of freedom; G – Germination (%); Tm – Mean time of germination (days); GSI – Germination speed index; **, *, ns Significant (p < 0.01), (p < 0.05) and not significant by F test, respectively

for limiting growth and yield of some species, especially for affecting primary processes, such as photosynthesis, synthesis of proteins, production of energy, and lipid metabolism (Flors et al., 2007).

The results of the analysis of variance for the evaluated variables suggest that the seeds were already in the process of deterioration, even maintaining high germination at 16

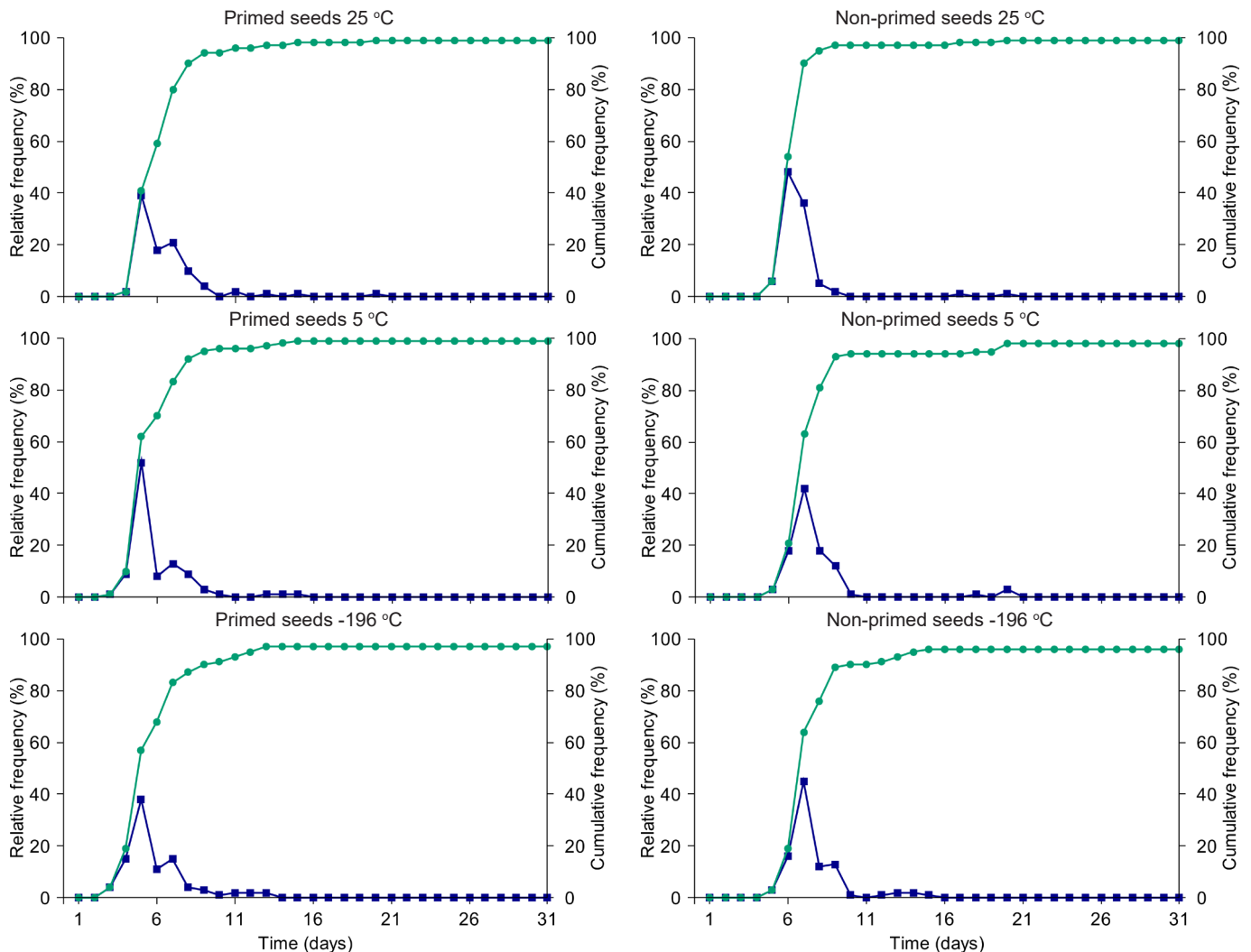


Figure 1. Relative and accumulated frequency (%) of primed and non-primed *P. peruviana* seeds at different storage temperatures until 16 months and germination in water

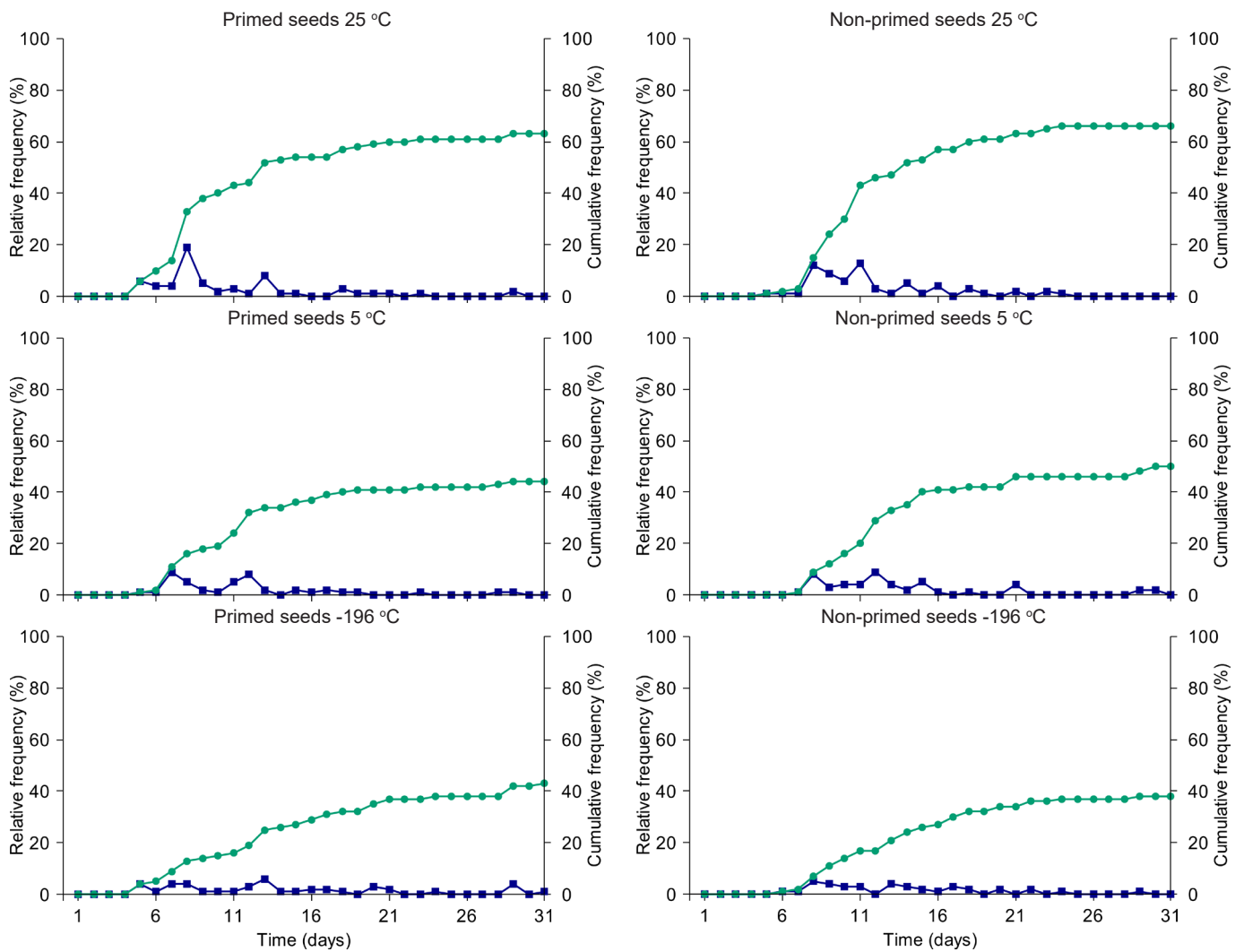


Figure 2. Relative and accumulated frequency (%) of primed and non-primed *P. peruviana* seeds at different storage temperatures until 16 months and subjected to salt stress

months. Souza et al. (2014a), carried out with recently collected, primed and non-primed seeds of *P. peruviana* under salt stress, obtained germination very close to the control (distilled water), of almost 100%, contrary to the result obtained in the present study, in which there was a decrease of germination to a range of 40-60% (Figures 1 and 2).

The polygons of relative and accumulated frequency for seeds primed and non-primed in water (Figure 1) and saline solution (Figure 2) showed sharp decrease in germination percentage for the latter, regardless of the type of seed and storage environment. It was observed that the polygons are unimodal for the seeds in water and polymodal for those in saline solution. The seeds in saline solution require more time to germinate, which also reflects in the uniformity of the process; however, osmoconditioned seeds maintained in water germinated faster than non-primed seeds, regardless of the temperature, which was evidenced by the displacement of the germination peak to the left of the graph (Figure 2). Souza et al. (2011) also observed that primed *P. angulata* seeds showed higher germination speed and uniformity.

The mechanisms of tolerance of seeds subjected to salinity depend on the ability of the protoplasm to compartmentalize the ions that enter the cell, because most of them accumulate in the vacuoles and many modifications occur in plant metabolism induced by salinity (Heuer, 1997). Among these,

there are modifications in the ionic balance, stomatal behavior and photosynthetic efficiency, water deficit and nutritional imbalance (Heuer, 1997).

Therefore, there was no difference between the seeds stored at 5 and -196 °C, and studies with seed storage longer than 16 months may answer whether cryopreservation would be more indicated than the temperature of 5 °C to delay seed deterioration.

CONCLUSIONS

1. Under low relative air humidity conditions, *P. peruviana* seeds can be stored for up to one year at 5, 25 and -196 °C.
2. For seed conservation for longer periods (greater than 12 months), low temperatures are recommended.
3. Previous priming can be a technique used to delay the deterioration of seeds stored for long period.

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