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Cryopreservation of tropical plant germplasm
-current reserch progress and applications -

PROGRAMME & ABSTRACTS

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P-15 Effects of moisture content on passiflora seed viability after immersion in liquid nitrogen

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Seed moisture content (MC) is probably the most critical factor in the definition of a successful cryopreservation protocol. As it relates to the composition of the seed, this implies a precise level of desiccation and tolerance to LN, species wise. Although the *Passiflora* genus comprises about 350 species and 22 subgenera in the Neotropics, very few species are important for fruit consumption, hence little research has been done to define its behaviour and storability and much less on cryopreservation.

Seed viability based on 2,3,5-Triphenyl-2H-tetrazolium chloride (TTC) and germination were tested to propose levels of desiccation that these species can tolerate as well as moisture content levels at which cryopreservation can be done.

Data suggest that *Passiflora* seeds tested can tolerate desiccation to about 10.0% MC wb; however, each species has individual levels of tolerance. As expected, none of the species tested was able to withstand 1-h immersion in LN at high moisture levels (15.0%). There was a gradual increase of germination when seeds reached the MC of 8.0% and progressive decrease with lower MC values. A suggested safe critical value was about 9.0% MC for *P. ligularis* and about 11.0% MC for *P. edulis*.

About germination, method of thawing did not contribute to the variation of the values, implying the effect was greatly due to the drying system and the species involved. Because of the lack of well-defined patterns of viability, results based on TTC can be misleading. It is suggested that experiments of this type be complemented with germination rather than TTC reduction tests alone.

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P-16 Cold acclimatization improves the cryopreservation of *in vitro* grown *Pyrus* and *Rubus* meristems

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Cold acclimatization (CA) is effective in improving regrowth of some cryopreserved tissues. Our objective was to determine the effects of CA on the cryopreservation of *Pyrus* and *Rubus* meristems. *In vitro* grown plants of these genera were cold-acclimatized with 22°C 8-h days/–1°C 16-h nights for 1 week or more and slowly frozen to –40°C before immersion in LN. CA improved regrowth for most of the genotypes tested but the magnitude of the response varied with genotype and growth conditions. The regrowth of meristems of *P. koehnei* Schneider and three other genotypes exceeded 75% after 1 week of CA. For *P. communis* L. cv. Beurre Bosc, *P. pashia* Ham., and the interspecific hybrid *P. communis* x *P. pyrifolia* (Burm. F) Nakai cv. Good Christian, 1 week of CA was less effective and the survival significantly increased after 4 or 6 weeks of CA. One to three weeks of CA did not improve survival of *P. cordata* Desv. CA for up to 8 weeks did not negatively affect many of these pear genotypes. Shoot regrowth of *Rubus*

EFFECTS OF MOISTURE CONTENT ON PASSIFLORA SEED VIABILITY AFTER IMMERSION IN LIQUID NITROGEN

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INTRODUCTION

Liquid nitrogen (LN_2) storage is certainly a very promiseful technique for long-term conservation, and its advantages (e.g. low maintenance costs, suspension of aging and postponing regeneration) have motivated the development of cryopreservation protocols for seed of many economically important species (Stanwood and Roos, 1979; Stanwood, 1985). The seed moisture content (M.C) is probably the most critical factor towards the definition of a successful cryopreservation protocol. As it relates to the composition of the seed, its storage behaviour and desiccation sensitivity, imply precise levels of desiccation and tolerance to LN_2 by species. Although several species of Passiflora (Passifloraceae; passion fruit) are important for fruit consumption, little research has been done to define its behavior, storability and much less on cryopreservation (Ellis et al., 1985).

The objectives were to define ranges of tolerance and critical points for seed moisture through different methods of drying and to compare the effect of the cryopreservation on seed viability of Passiflora edulis and P. ligularis at different seed moisture contents.

MATERIALS AND METHODS

Seed source and conditioning

Ripen mature fruits from Passiflora edulis Sims f. flavicarpa Degener and P. ligularis Juss. were obtained from a farmer source and selected for similar size and weight. At the laboratory, for conditioning, seeds were washed after aril extraction, then classified by density in water, discarding the light fraction.

Desiccation and moisture content

Four moisture content levels were obtained by drying under three groups of conditions:

- Eight hours at laboratory conditions (22°C and 40-50% air relative humidity (R.H))
- Five days at laboratory conditions (22°C and 40-50% R.H)
- Five days in the drying room (22°C and 20-30% R.H)
- Two days over silica gel (22°C and 5-13% R.H). Seeds were placed over a small screen in a sealed plastic tray containing 80 g of silica gel each.

After each desiccation period, seeds were removed for evaluation of germination, moisture content and cryoexposure.

Moisture content was determinated gravimetrically in an oven at 130 °C for 1 hour (ISTA, 1993) and expressed as percentage of fresh weight basis (f.w.b) with three replicates of 50 seeds.

Cryoexposure of Passiflora seeds

Seed at different moisture contents were packed in vacuum sealed trilaminar foil bags and directly dipped into LN_2 held in a styro-foam cooler during 1hour (fast cooling). After cooling and before planting for germination, the seeds were thawed by two different methods:

- Slow thawing (STHAWING): Seed attained the laboratory temperature

➢ Fast thawing (FTHAWING): Sealed bags with seed were immersed in a water bath set at 37°C, during 1 minute.

Evaluation

- Germination test

Germination tests were undertaken on three replicates of 100 seeds after applying treatments to break dormancy (Ospina et al., 1998). For P. ligularis, seeds were soaked in a GA_3 solution at 500 ppm (24 h). The solution was then removed and the seeds left for 48 hours before finally soaked in sterile water during 24 h. Next, the seeds were placed in rolls of standard germination paper under laboratory temperature (22°C). For Passiflora edulis, seeds were soaked in sterile water during 24 h, then the rolls were incubated in a germinator at 35 °C /25 °C (8/16 hours), with 8 hours of light period. At the beginning of the test, the paper was wetted with a fungicide solution of Banrot 1% to avoid growth of saprophytic fungi. Evaluations were recorded during four weeks.

- **TTC test**

Before examining the seeds from the different treatments and since there were no standard patterns for testing viability with 2,3,5- Triphenyl-2H-tetrazolium chloride (TTC) on Passiflora seed, those were previously defined (Figure 1d) (Ospina et al., 1998).
Seed was preconditioned for the test: nicking a small piece of seed coat on the distal embryo region, fracturing the seed coat with the aid of pliers and detaching at least 50% of it (Figures 1a,b,c). Then the seeds were immersed in the 0.5% (w/v) TTC solution during 24 hours and finally were rinsed and evaluated according to the pattern.

Since dormancy was not fully overcome at the end of the germination test, the viability test was done on recovered seeds (non germinated seeds), so, the evaluation of all treatments were made using the criteria of total viability (GERM+TTC) by adding to the normal seedling, those non germinated but viable seed according to the TTC test.

RESULTS AND DISCUSSION

General results for moisture content and total viability of seeds of P. edulis and P. ligularis are given in Table 1.

Effects of drying

After seed extraction and conditioning, initial M.C for P. edulis and P. ligularis were 21% and 30%, respectively. Those values were reduced after reaching equilibrium points and depending on the drying method and the species. After 8 hours at the laboratory, the equilibrium M.C. for P. edulis and P. ligularis corresponded to 14.4% and 16.7%, while after 5 days at the laboratory were 7.7% and 8.8%, respectively. At the drying room, the species reached 5.8% and 5.9%, while seed that was exposed to the silica gel showed more differences in their equilibrium point 2.6% and 4.0%, respectively (Table 1).

Independently of the drying condition, P. ligularis being more hygroscopic reached slightly higher values than P. edulis suggesting particular differences in composition by species (Table 2).

In relation to tolerance of drying, total viability showed that both species were affected by lowering their M.C. For P. edulis, total viability was reduced from 94.3% to 79.0% once M.C. dropped from 14.4% to 7.7% and up to 57.0% with M.C. of 2.5 %. On the other hand, P. ligularis reduced its total viability from 97.6% to 89.0% once seed M.C. dropped from 16.7% to 7.9% and up to 81.3% if M.C was 4.0% (Table 1).

The drying sensitivity observed in P. edulis, confirm its intermediate behaviour, as before suggested by Hong, 1996, while the lesser extent by P. ligularis suggests a more orthodox behaviour. Intermediate seeds cannot withstand drying below 9.0% or if they can be dried further down, they cannot withstand storage at temperatures lower than –40°C (Stanwood, 1985)

Effect of cryoconservation

Independently of the thawing method and at M.C of 14.4% and 16.8% for P. edulis and P. ligularis, respectively, lethal values (0%) of germination and total viability were reported (Table 1). This suggests that these M.C. are well above any high moisture freezing limit (HMFL) for these species.

On the other hand, the higher values for total viability (72% and 89.3%) were obtained by P. edulis and P. ligularis respectively at 7.7 % M.C and 8.0% M.C, implies a safe freezing M.C by species. Considering the M.C at which higher values were observed it is suggested that P. edulis has a safe freezing M.C around 11%, while P. ligularis presents this around 9.0%. These differences also relates to the drying sensitivity, and being P. edulis more sensitive to drying, showed higher safe M.C than P. ligularis.

For the two species and in the majority of M.C levels, the fast thawing method resulted in higher outputs than the slow thawing, the former being more recommended for a cryo protocol of these species.

Table 1. Effect of cryoconservation at two different thawing methods for P. edulis and P. ligularis seeds

SPECIES	Drying Condition (time)	M.C (%)	TOTAL VIABILITY (%)		
			No - Cryo	Cryo STHAWING	Cryo FTHAWING
Passiflora edulis	L.C (8h)	14.4	94.3	0.0	0.0
	L.C (5d)	7.7	79.6	72.0	51.0
	D.R (5d)	5.8	77.6	60.0	67.3
	S.G (2d)	2.6	57.0	50.6	55.3
Passiflora ligularis	L.C (8h)	16.8	97.6	0.0	0.0
	L.C (5d)	8.0	89.6	79.3	89.3
	D.R (5d)	5.9	86.0	53.6	60.0
	S.G (2d)	4.0	81.3	56.0	68.0

L.C= Laboratory conditions, D.R= Drying room, S.G= Silica gel

Table 2. Analysis of variance procedure

Source of Variation	M.C. (%)	Total viability (%)
Species	*	**
Drying condition	**	**
Method of thawing	-	N.S
Specie x Drying condition	N.S	**
Specie x Method of thawing	-	**
Drying condition x Method of thawing	-	**
Specie x Drying condition x Method of thawing	-	**

** = High significance (Pr<0.01), * = Significance (0.01<Pr<0.05), N.S = No significance (Pr > 0.05)

CONCLUSIONS

- Data suggest that the Passiflora seeds tested can tolerate desiccation to about 10.0% M.C (f..w.b), however, each species has individual levels of tolerance. The sensitivity of P. edulis under drying confirm its intermediate behaviour, as suggested by Hong et al., 1996; to a lesser extent, the sensivility of P. ligularis seggests a more orthodox.

- As expected, none of the species tested were able to stand 1-hour immersion in LN_2 at high moisture levels (15.0%). The higher total viability values obtained around 8% for both species and a progressive decrease with lower M.C values. It is also suggested that safe critical value for P. ligularis is about 9.0% M.C, while for P. edulis is about 11.0% MC.

- For species with dormant seed as the ones used in this experiment, it is necessary to carry out some previous work for the definitions of germination pretreatments and patterns of viabilty using TTC.

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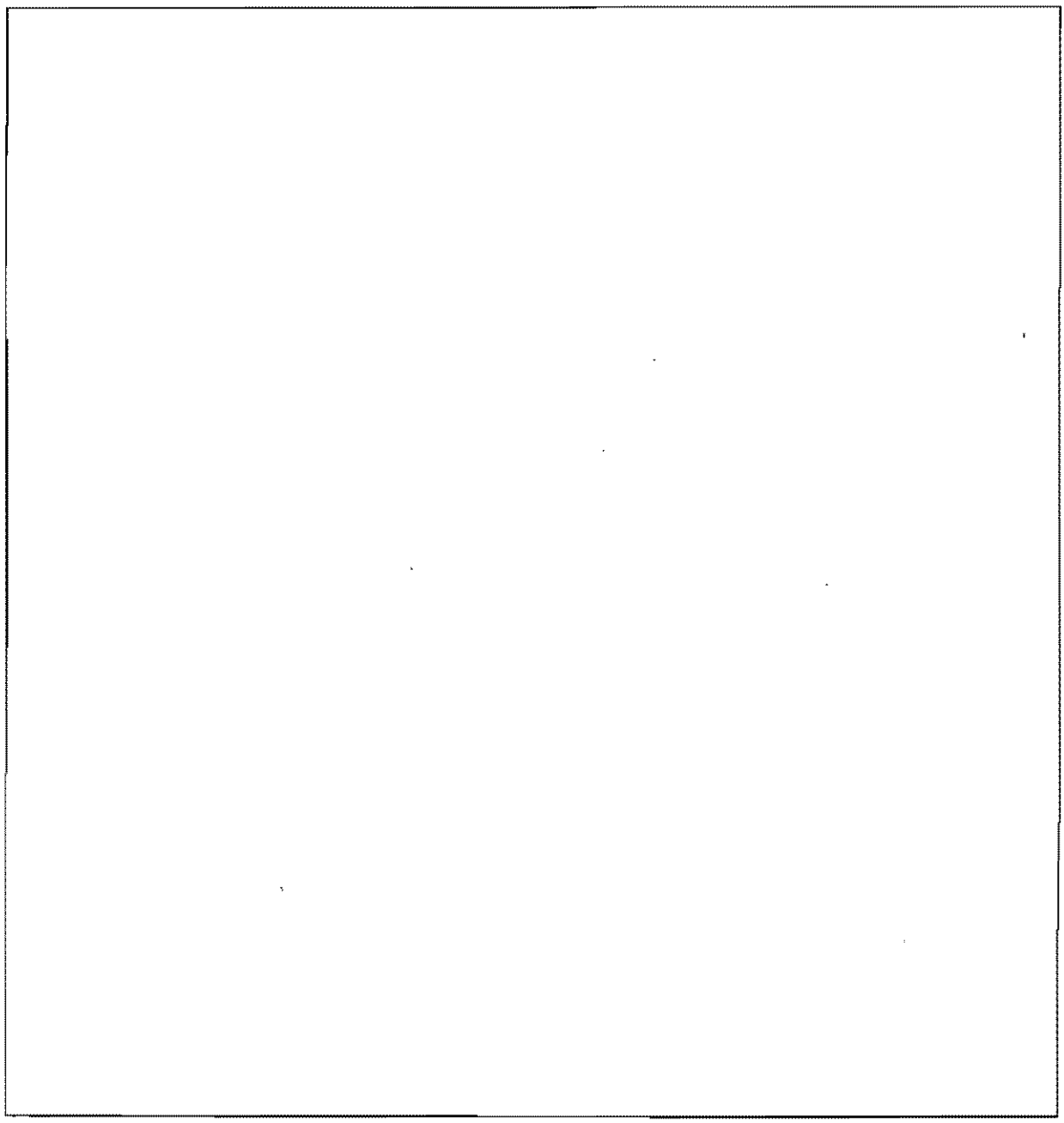
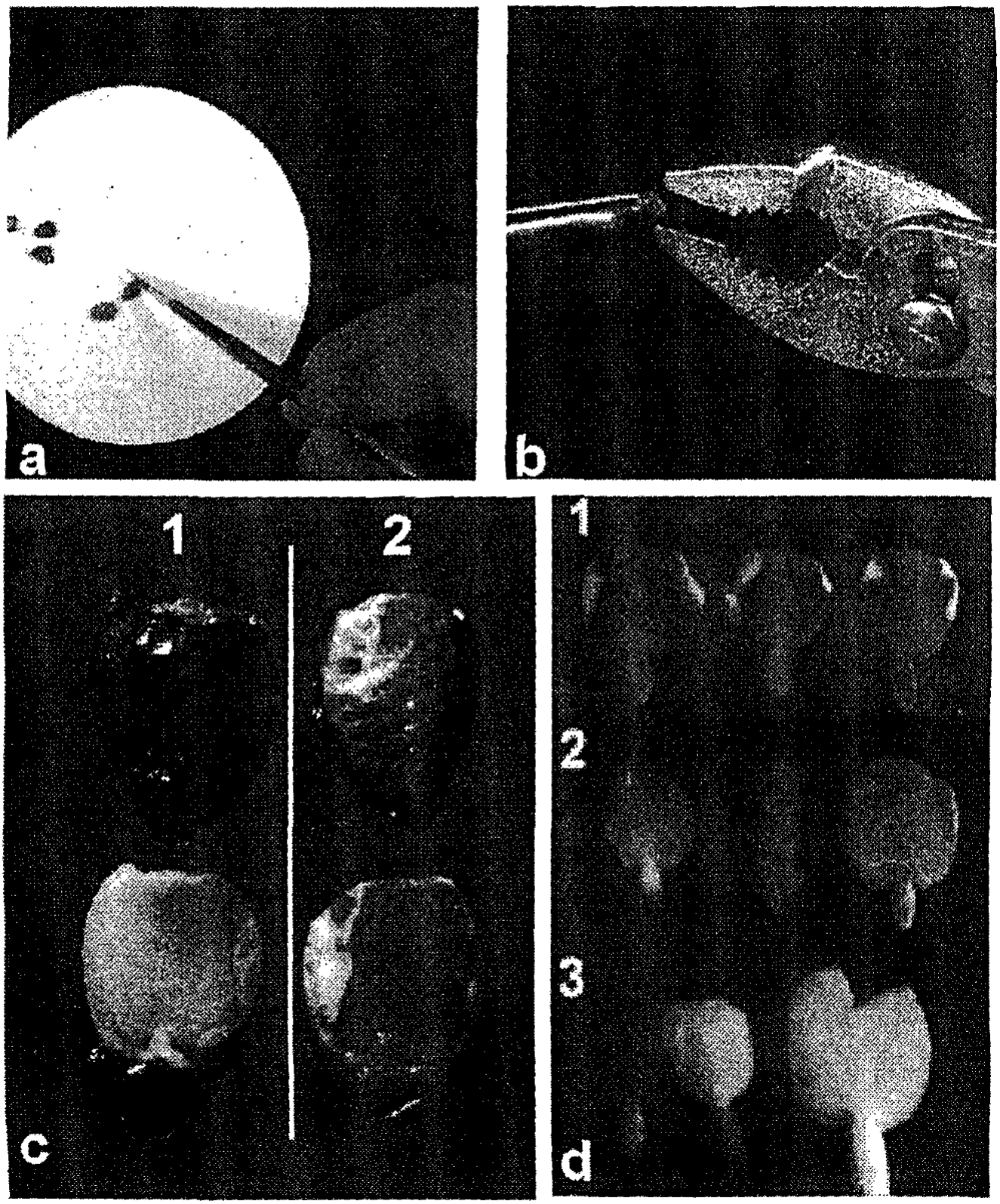
Figure 1. **Conditioning and evaluation of viability of Passiflora seeds by TTC method**



a- Nicking a small piece of seed coat on the distal embryo region
b- Fracturing the seed coat with pliers
c- Exposing to TTC 1- Whit nick alone, 2- Whit nick and detaching at least 50% of seed coat
d- Patterns: 1- Viable, 2 and 3- Non viable

Ospina et al., 1998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