



Figure 1. Morphological characteristics of ulluco. Source: Manrique et al. 2017

METHODOLOGY

- A diploid clone of ulluco ($2n = 2x = 24$) was used to carry out the experiment.
- 28 days pre-treatment on $\frac{1}{2}$ MS (Murashige & Skoog 1962) under a 16/8 h light/dark regime at 17 °C.
- Transferred to $\frac{1}{2}$ MS medium supplemented with mannitol (10-30 g l⁻¹), sorbitol (10-30 g l⁻¹), sucrose (10-120 g l⁻¹), chlorocholinchlorid (CCC; 300-700 mg l⁻¹) or abscisic acid (ABA; 1-3 mg l⁻¹). 18 treatments including control.
- Cultivation conditions: 4 °C and 24 h/dark for the 24-month.
- Microtuber (MT) recovery after conservation: Three culture media were tested MS, $\frac{1}{2}$ MS and MS supplemented with 0.5 mg l⁻¹ GA3 for 3 months under a 16/8 h light/dark regime at 17 °C.
- Statistical analysis performed using one-way ANOVA and Turkey's HSD test ($P \leq 0.05$) [StatSoft STATISTICA 12.0].

Table 1. Effect of recovery medium on MT regeneration after conservation

Reg. media	Conservation treatment	Plant survival	No. of shoot/plant (mean ± SE)	Shoot height (cm) (mean ± SE)	No. of leaves/plant (mean ± SE)
$\frac{1}{2}$ MS	Control	100%	1.20±0.2abc	1.48±0.1a	6.10±1.3ab
	Mannitol 20 g l ⁻¹	80%	1.10±0.2abc	2.20±0.4ab	4.40±0.9ab
	Sorbitol 30 g l ⁻¹	100%	1.00±0.0abc	1.36±0.0a	3.60±0.3a
	Sucrose 90 g l ⁻¹	100%	1.10±0.2abc	1.87±0.2a	6.70±1.1b
MS	Control	80%	1.10±0.3abc	1.73±0.4a	4.70±1.3ab
	Mannitol 20 g l ⁻¹	70%	0.70±0.2a	1.83±0.5a	3.30±0.9a
	Sorbitol 30 g l ⁻¹	100%	1.10±0.1abc	1.36±0.1a	3.30±0.4a
	Sucrose 90 g l ⁻¹	90%	1.90±0.4c	1.96±0.4ab	6.80±1.5b
MS+0.5 mg l ⁻¹ GA ₃	Control	90%	0.90±0.1ab	1.42±0.2a	3.50±0.6a
	Mannitol 20 g l ⁻¹	60%	1.40±0.7abc	1.56±0.5a	5.00±1.9ab
	Sorbitol 30 g l ⁻¹	100%	1.80±0.0bc	1.69±0.0a	5.10±0.3ab
	Sucrose 90 g l ⁻¹	100%	4.30±0.4d	2.82±0.1b	11.70±0.4c

Table 1. Mean followed by different letters, were significantly different according to Tukey's HSD test ($P \leq 0.05$). SE standard error.

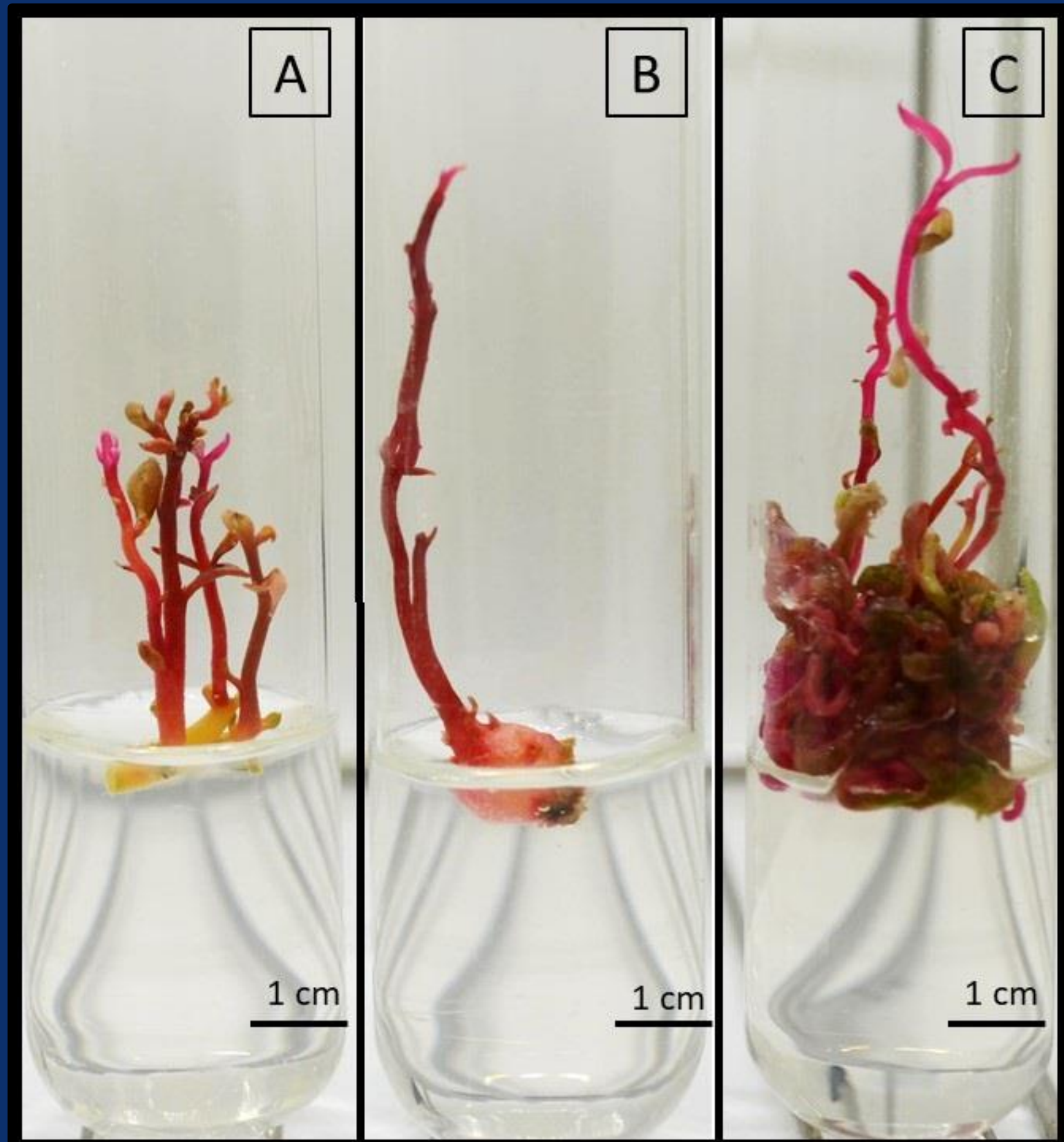


Figure 4. Germination of MT produced by plantlets conserved on sucrose 90 g l⁻¹. On A) MS, B) $\frac{1}{2}$ MS or C) MS+0.5 mg l⁻¹ GA₃ as recovery media.

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In vitro conservation and recovery of *Ullucus tuberosus* after reduced growth of microshoots

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- Ulluco (*Ullucus tuberosus* Caldas) originated in the Andes
- Staple crop for Andean communities
- It produces edible tubers rich in carbohydrates and vitamin C
- The species has a high morphological (Fig. 1) and genetic diversity
- Vegetatively propagated crop - seed and *in situ* conservation are difficult to perform, creating the need to develop alternative biotechnological methods for ulluco germplasm conservation

Study Objective: To develop a reliable medium-term conservation protocol for ulluco by testing various slow-growth treatments and regeneration.

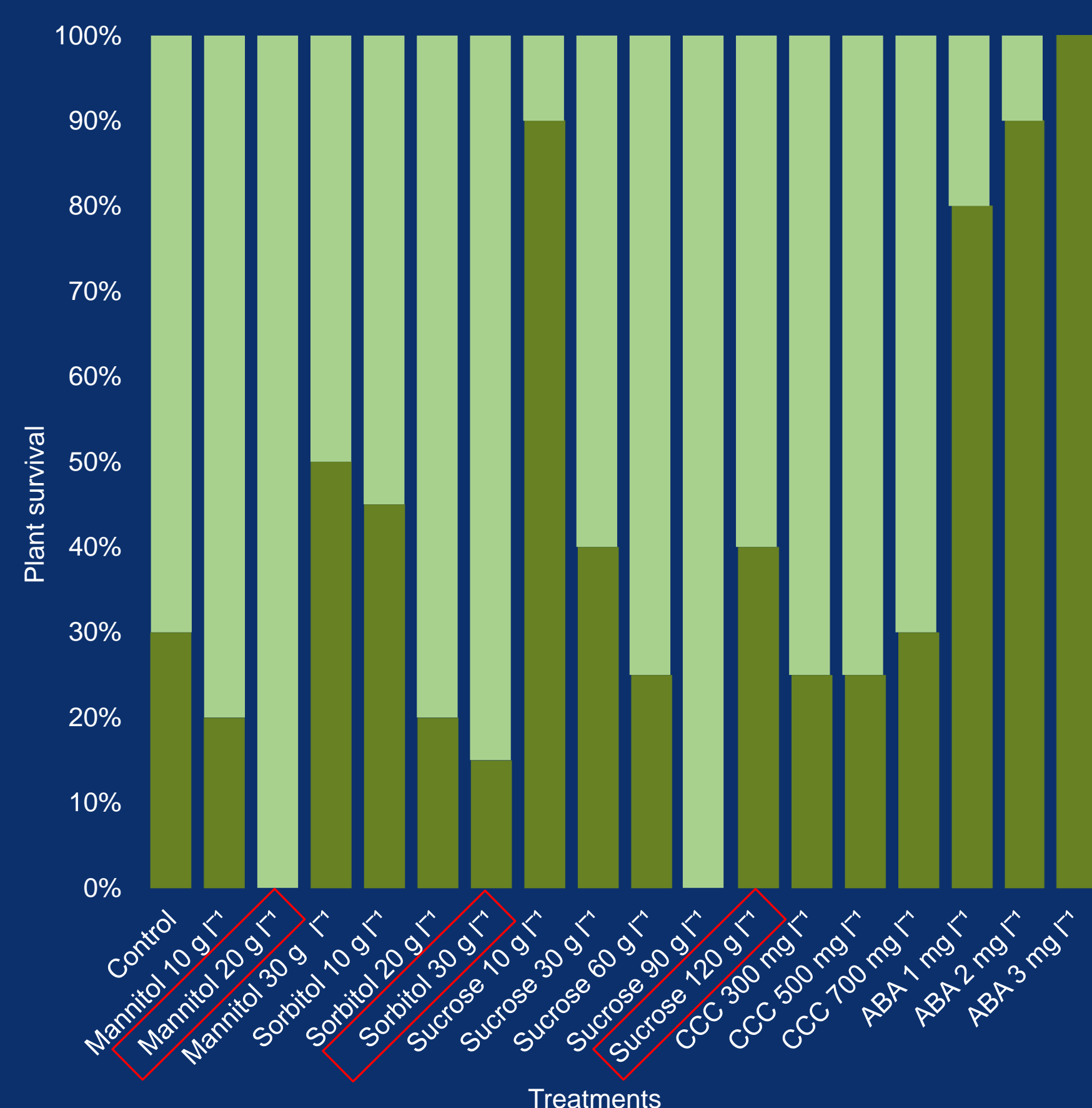


Figure 2. Survival rate of ulluco plantlets after a 24-month conservation on various growth reduction treatments.

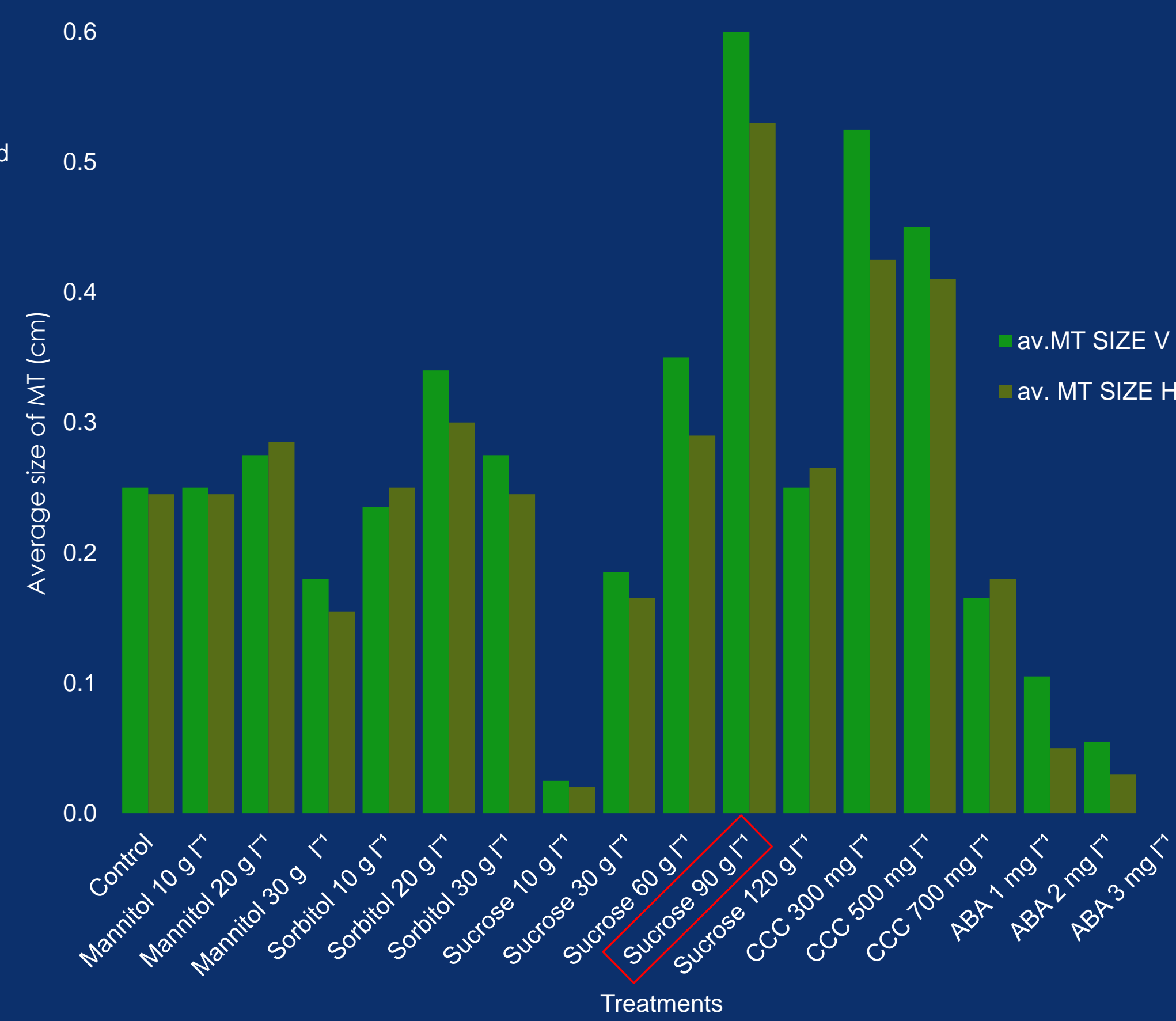


Figure 3. Average (av) size of microtubers (MT). V=vertical, H=horizontal.

RESULTS

- Sucrose 90 g l⁻¹ and mannitol 20 g l⁻¹ provided best survival results (100%) after conservation followed by sorbitol 30 g l⁻¹ (85%) (Fig.2).
- CCC also proved to have inhibitory effect on the growth of plantlets but was not as effective (Fig.2).
- Survival after conservation was mainly in the form of MTs. 90 g l⁻¹ sucrose produced the largest MTs (Fig.3).
- ABA treatments proved to be inadequate for ulluco conservation providing a maximum survival rate of 20% after conservation (Fig.2).
- The best recovery media proved to be MS supplemented with 0.5 mg l⁻¹ GA₃ (Fig. 4)
- MTs produced by plantlets on 90 g l⁻¹ sucrose treatment during conservation had the best recovery after conservation. Providing a 100% survival and showed the best recovery in evaluated parameters (Table 1).

CONCLUSION

- Ulluco can be stored for 24 months with survival rates of up to 100% in form of microtubers.
- The tested treatments sucrose (90 g l⁻¹), mannitol (20 g l⁻¹) and sorbitol (30 g l⁻¹) are most effective in reducing ulluco growth while maintaining the vitality of the plant at 4 °C, under 24 h/dark regime.
- MS+0.5 mg l⁻¹ GA₃ is the most optimal recovery media for MTs germination after conservation.
- 90 g l⁻¹ sucrose is the most effective treatment over all in terms of conservation and recovery.
- The optimized slow-growth treatment for ulluco represents a reliable alternative to safeguard this vegetatively propagated crop.

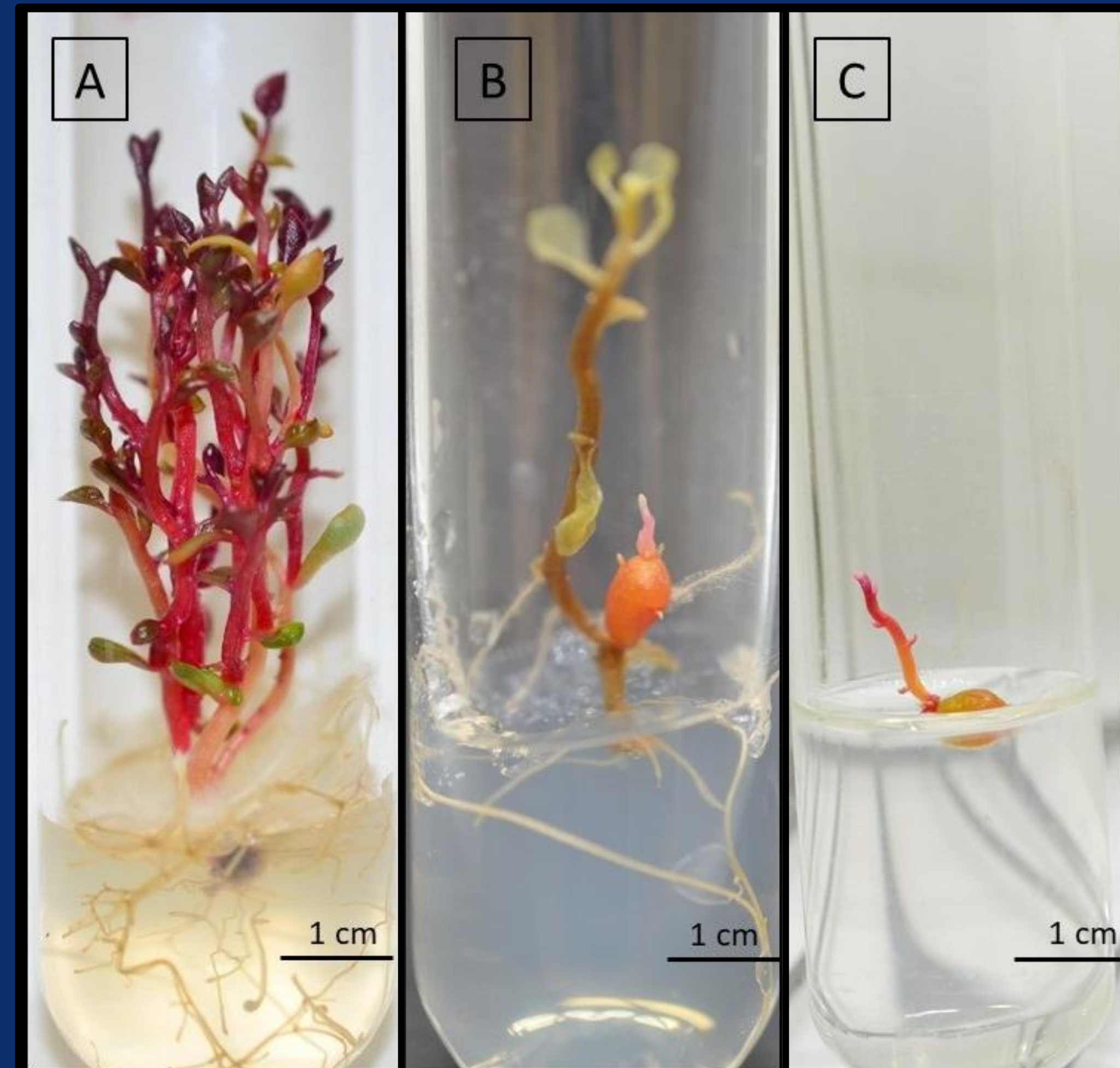


Figure 5. Phased of slow-growth. A) initial plant material, 1-month pre-treatment, B) plantlet after 24-month conservation and C) germination of MT on recovery media.