# Cryopreservation and virus elimination of shallot by droplet-vitrification

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## Introduction

- Shallot is an important vegetable belonging to the genus *Allium.* It possesses rich sources of flavonoids and high level of antioxidant activity and is a heathy dietary option for humans.
- Vegetative propagation makes production of shallot heavily affected by virus infection. Therefore, use of virus-free shallot propagules is pivotal for sustainable shallot production.
- Cryopreservation is a reliable method for long-term conservation of shallot plants.

## Material

Shallot '10603' maintaining on MS + 30g sucrose/L + 6-BA 0.5mg/L + NAA 0.1mg/L

## Cryopreservation

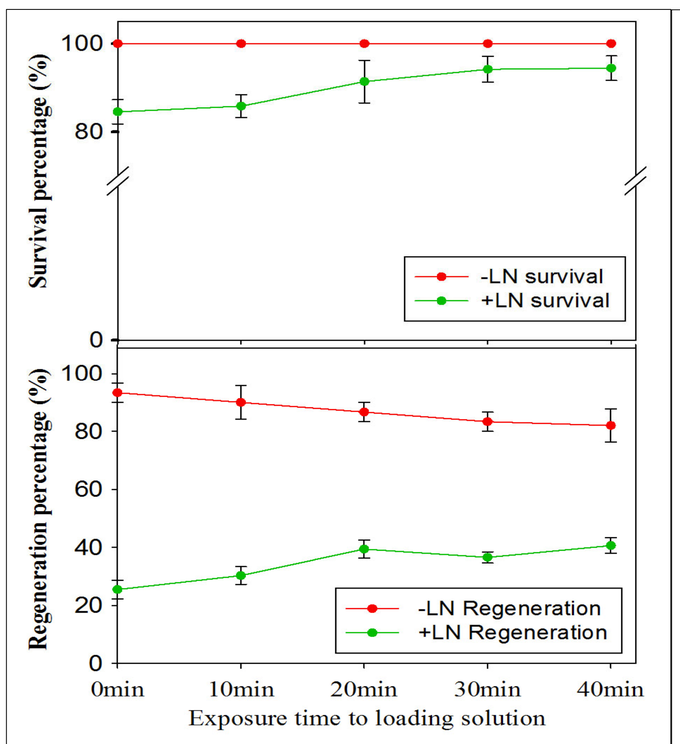



**Fig. 1** Effects of different durations of loading treatment on survival and shoot regrowth of cryopreserved shoot tips of shallot '10603'

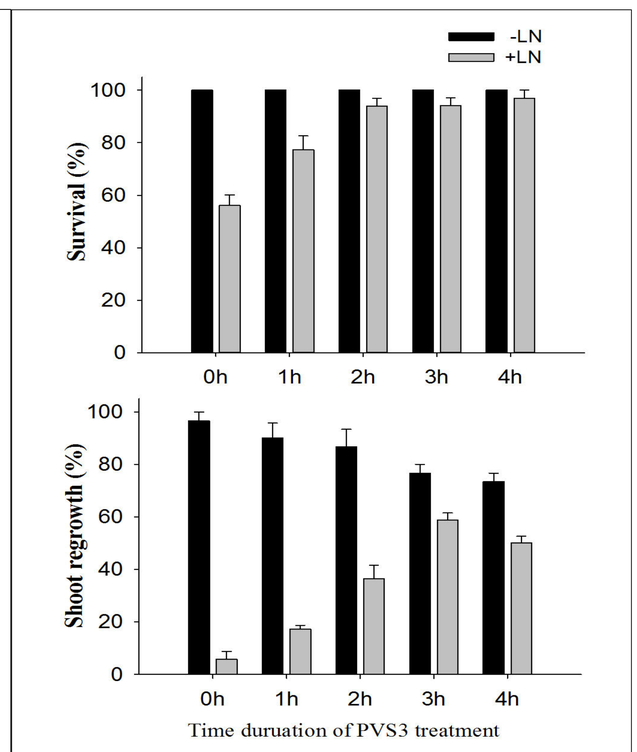



**Fig. 2** Effects of different durations of PVS3 treatment on survival and shoot regrowth of cryopreserved shoot tips of shallot '10603'

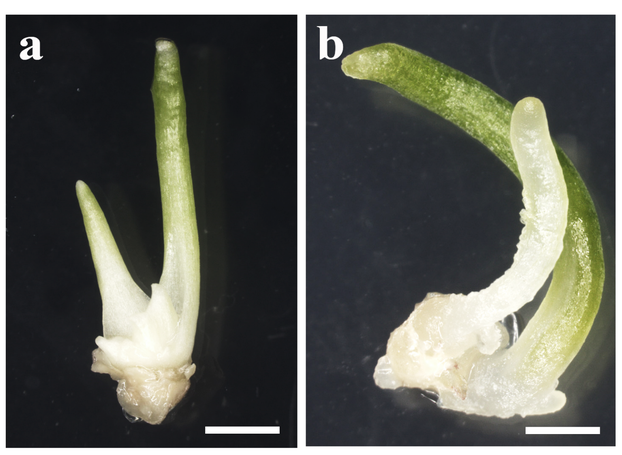
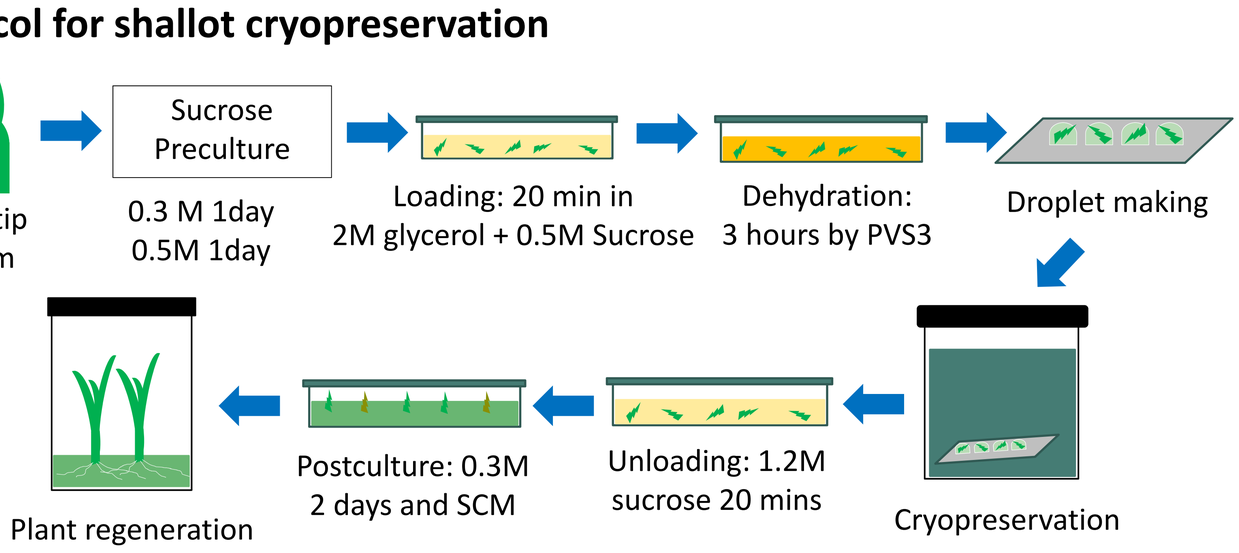



**Fig 3**. Shallot shoot after cryopreservation; a, non-hyperhydrated shoot; b, hyperhydrated shoot. bars = 1.0 mm

## Postculture

When postculture was only done on subculture medium (SCM) for regeneration, the regrowth rate after cryopreservation was 36% and 45% of regenerated shoot tips were hyperhydrated. When cryopreserved shoot tips were transferred to sucrose enriched medium either at 0.5M followed by 0.3M or 0.3M for two days, more than 50% percentage of regrowth rate could be obtained and hyperhydration rates decreased significantly compared with direct post culture on SCM.

## Protocol for shallot cryopreservation




## Virus detection, localization and elimination

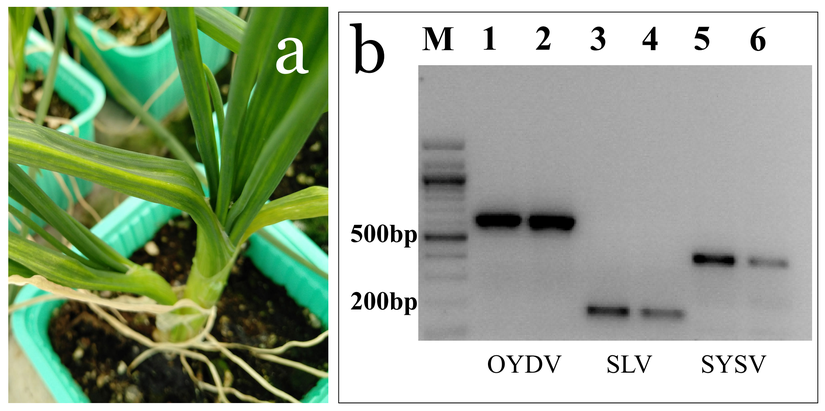



**Fig. 4** a, Virus symptoms of shallot '10603' growing in the greenhouse. b, Virus detected from shallot '10603' by RT-PCR. M, 100 bp DNA ladder; 1, 2 samples detected by Onion yellow dwarf virus (OYDV) primer; 3, 4 samples detected by Shallot latent virus (SLV) primer; 5, 6 samples detected by Shallot yellow stripe virus (SYSV) primer.

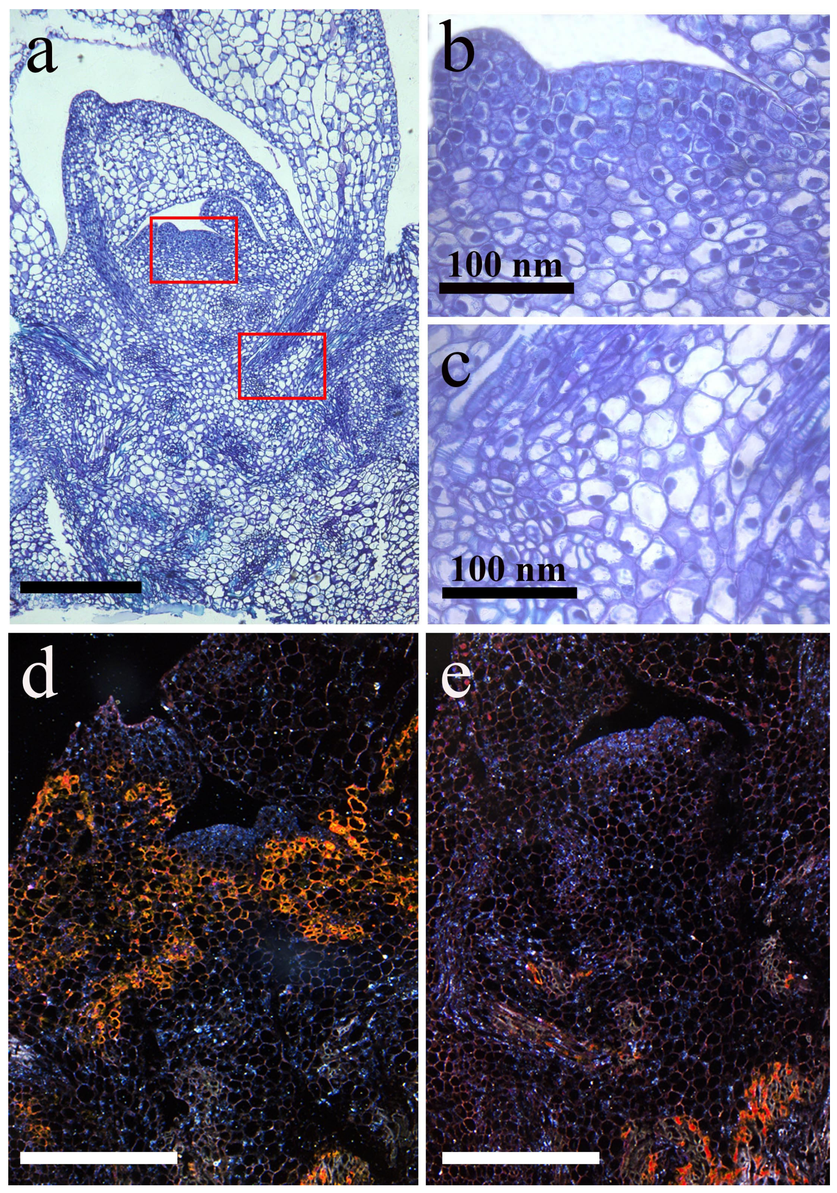



**Fig. 5** Histological observation and virus immunolocalization of shallot shoot tips '10603' after cryopreservation. a, longitudinal section of shoot tip after cryopreservation. b and c, amplified areas of red squares in 'a' showing cells in both meristem and stem disc tissue survived after the cryopreservation. d and e, immunolocalization of OYDV (d) and SLV (e) in shallot shoot tips. Virus coat protein was localized and showed orange color. Bars without numbers = 0.5 mm.

**Table 1**. Effects of cryopreservation on virus elimination in shallot

| | Frequency of virus eradication | | |
|---|---|---|---|
| Virus cleaning procedure | OYDV | SLV | SYSV |
| Shoot tip culture | 0/10 | 0/10 | 0/10 |
| Cryotherapy | 1/13 | 2/13 | 1/13 |

## Conclusion

Cryopreservation of shallot is successfully achieved with a regrowth rate higher than 50% after sufficient dehydration in PVS3 solution. However, the optimized cryo-preservation protocol leads to the poor efficiency of virus eradication as both cells from the meristem and stem disc survived after the cryopreservation.

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