

**Poster**

**CRYO PRESERVATION OF *IN VITRO* DALMATIAN  
CHRYSANTHEMUM SHOOT TIPS BY DROPLET VITRIFICATION  
METHOD**

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**ABSTRACT**

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is the most common and family flower for Japanese people. Although, cryopreservation is the most suitable method to conserve it, a successful protocol has not been developed for conservation of chrysanthemum. Consequently, a cryogenic protocol was tested by droplet vitrification method to identify; optimal shoot tips exposure period into PVS2 solution, different pre-culture periods and media, optimal cool hardening period, and with different plant vitrification solutions (PVS2, PVS3, and PVS4) and different varieties. Shoot tips were excised from the two weeks cool hardened *in vitro* Dalmatian Chrysanthemum plantlets under the microscope in the aseptic conditions. These shoot tips were cultured on MS medium supplemented with 0.3 M sucrose (0.2 mg/l BA + 8 g/l Agar) and, pre-cultured in an incubator (5 °C, and 8 hours photoperiod under white fluorescent light, 26 µmol /m<sup>2</sup>/s) for 3 days. After pre-culture, the shoot tips were used for the experiment. Results indicate that *in vitro* shoot tips of the Dalmatian Chrysanthemum can be successfully cryopreserved with 70% survival rate in droplet vitrification method when viable *in vitro* shoot tips are used with, 14 days cool hardening, 16 hours light (light intensity- 52 µ/cm<sup>2</sup>/s) and 8 hours dark condition, 5 days pre-culture period under 8 hour light(light intensity- 26 µ/cm<sup>2</sup>/s) and 16 hour dark condition, osmoprotected with LS solution for 20 minutes and dehydration by PVS No 4 for 50 minutes under 25 °C. The droplet verification appears as a highly valuable and an efficient method for cryopreservation of Dalmatian Chrysanthemum.