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Establishment of an Efficient *In Vitro* Culture System in *Dicentra* × *hybrida*

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Abstract: *Dicentra* × *hybrida* is a popular ornamental hybrid, cultivated in parks and gardens worldwide. To date, there are no reports on establishing a plant tissue culture system in this species. This study aimed to develop an efficient micropropagation protocol in *D.* × *hybrida* 'Amore Rose' for further reproduction and breeding. Shoots of *in vivo*-grown plants were dissected and washed with running tap water. Next, shoot segments were bathed in tap water with a drop of detergent for 30 min and, then, immersed in 70% (*v/v*) ethanol for 1 min. Following the initial disinfection, explants were surface sterilized with 0.4 – 0.8% (*v/v*) sodium hypochlorite (NaOCl) for 20 min and, finally, washed thrice with sterile distilled water (20 min each). The rosette explants were inoculated polarly in the modified Murashige and Skoog medium devoid of plant growth regulators and cultured in a growth room with a 16-h photoperiod. After one month, contamination-free explants were transferred on the kinetin-supplemented medium. The disinfection efficiency reached 10 – 40%. All disinfected explants were capable to develop healthy shoots. The multiplication ratio, i.e. the number of secondary explants produced reached up to 6.0. All developed shoots regenerated roots spontaneously. The obtained plantlets were successfully acclimatized to *ex vitro* conditions.

Keywords: axillary bud; bleeding heart; disinfection; micropropagation; ornamental plants; sodium hypochlorite

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Results

The results of the experiment are given in Table 1 and Figure 1:

Table 1. Efficiency of a micropropagation protocol in *Dicentra* × *hybrida* 'Amore Rose'.

NaOCl [%]	Axenic cultures [%]	Contamination		Shoot length [mm]	No. of leaves	Multiplication ratio	Rooting [%]	*Ex vitro* survival [%]
		Fungal [%]	Bacterial [%]					
0.4	10.0 a	90.0 b	0.0 a	70 a	44 a	6.0 a	100 a	100 a
0.6	20.0 a	60.0 b	20.0 ab	55 ab	34 a	5.0 a	100 a	100 a
0.8	40.0 a	20.0 a	40.0 b	40 b	10 b	2.0 b	100 a	100 a [1]

[1] means in columns followed by the same letter do not differ significantly according to Fisher's test at $P \leq 0.05$.

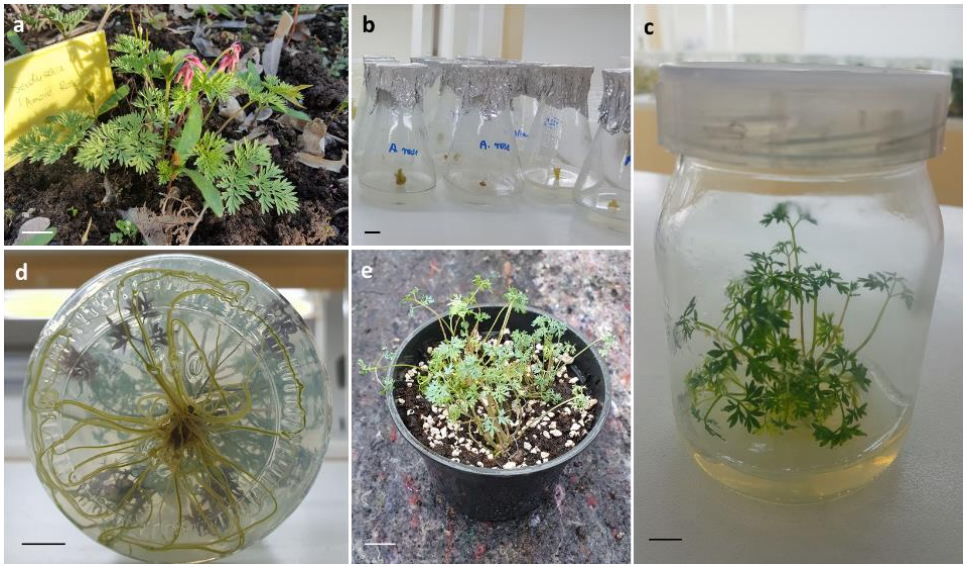

Figure 1. Micropropagation of *Dicentra* × *hybrida* 'Amore Rose': (**a**) flowering plants in the glasshouse; (**b**) primary explants in the PGRs-free MS medium; (**c**) fully developed microshoots of bleeding heart; (**d**) adventitious roots after two months of *in vitro* culture; (**e**) plantlets acclimatized to *ex vitro* conditions. Bar = 1 cm.

Results and Discussion

In *Dicentra*, all leaves are in a basal rosette, close to the soil/gardening substrate, which hinders the disinfection process. At the same time, cautious treatments are required not to damage the delicate meristems. This is the first report on surface sterilization of vegetative organs in *D.* × *hybrida*.

Based on the present observations (Table 1), it can be suggested that NaOCl is more effective in eliminating fungal microorganisms than bacterial ones. The reported decrease in shoot length, number of leaves, and multiplication ratio in bleeding heart treated with the highest concentration of NaOCl (0.8%) is probably a result of meristem damage during the exposition.

The present study demonstrated that *D.* × *hybrida* can be easily propagated *in vitro* even in a classical MS medium devoid of plant growth regulators (PGRs), although the addition of KIN, which activates the existing meristems, improves the development of microshoots.

Caulogenesis and rhizogenesis can be induced simultaneously in bleeding heart ‘Amore Rose’, without exogenous application of auxins, which is not the case with every plant species. The use of meristematic explant and lack of callus developmnt suggest maintaining trueness-to-type with the mother plant, although more studies are needed to confirm this.

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Results and Discussion

In the present study, all of the plantlets survived acclimatization to the greenhouse and maintained high quality without the need for an additional rooting step. This highlights the usefulness of the described here protocol in the large-scale reproduction of *D. × hybrida*.

Conclusions

This is the first complete report on the micropropagation of *Dicentra × hybrida*. The developed here protocol can be recommended for large scale reproduction of this species with possible extension to other members of the *Dicentra* genus. Further studies should focus on inducing adventitious organogenesis from leaf explants of the *in vitro*-derived plant material.