APPLICATION OF NANOPARTICLES IN CRYOPRESERVATION OF PLANT TISSUES

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INTRODUCTION

Cryopreservation, i.e. the storage of tissues in liquid nitrogen, is the safest form of biodiversity protection. Nanotechnology has the potential to enhance the efficiency of cryopreservation protocols through the acceleration of thermo-dynamic events in the cells. This study aimed to verify the usefulness of gold nanoparticles in plant cryopreservation.

Fig. 1. *Lamprocapnos spectabilis* 'Valentine' used in the study

MATERIALS AND METHODS

Gold nanoparticles (AgNPs) at various concentrations were used during various steps of the encapsulation-vitrification protocol for shoot tips of *Lamprocapnos spectabilis*. The influence of AuNPs on cryopreservation efficiency was determined by evaluating the recovery rate of explants and their morphogenic response; the membrane stability index (MSI); the concentration of pigments in shoots; and the antioxidant enzymes activity. The genetic stability of the plant material was evaluated using SCoT markers.

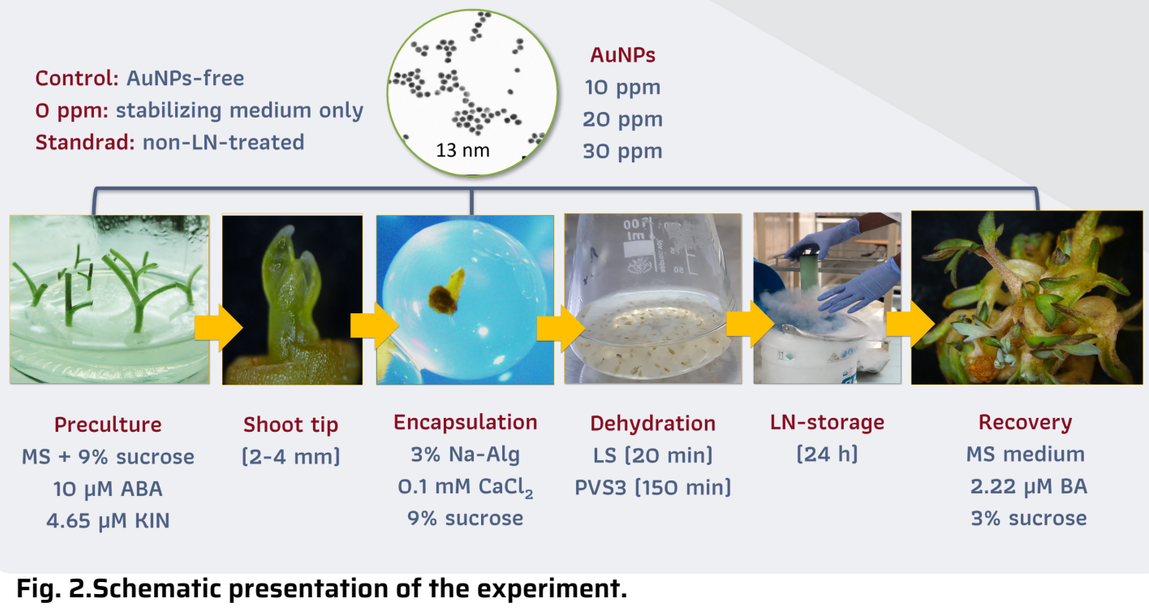

Fig. 2.Schematic presentation of the experiment.

RESULTS

It was found that 10 ppm of AuNPs added into the alginate bead matrix improved the recovery level of LN-derived shoot tips (70.0%) compared to the non-NPs-treated cryopreserved control (50.5%). On the other hand, the presence of nanoparticles in the recovery medium had a deleterious effect on the survival of explants.

Treatment	Recovery [%]	No. of shoots	Shoot length [mm]	Shoot FW [mg]	Shoot DW [%]	Rooting [%]	No. of roots
standard	100 a*	1.2±0.2 d	50.8±6.9 a	190.2±52.7 cd	11.6 cd	100 a	3.2±0.6 a
control	50.5 cd	2.3±0.2 a-c	26.2±4.3 bc	502.3±78.2 ab	13.1 cd	60.8 a-c	2.3±0.8 a-d
AuNPs in the preculture medium							
0 ppm	51.4 cd	2.2±0.1 a-c	21.0±2.9 c	381.9±86.9 b-d	14.9 b-d	51.7 b-d	1.1±0.3 c-e
10 ppm	61.7 bc	2.4±0.3 a-c	21.2±2.6 c	370.4±84.1 b-d	20.2 b	48.2 b-e	1.4±0.4 b-e
20 ppm	46.5 cd	2.3±0.3 a-c	22.1±3.2 bc	484.6±123.1 ab	16.6 bc	28.3 c-e	0.7±0.3 de
30 ppm	39.2 de	1.6±0.2 cd	16.0±1.3 c	169.3±38.9 d	12.1 cd	20.2 de	0.5±0.3 de
AuNPs in the protective bead matrix							
0 ppm	48.0 cd	2.3±0.2 a-c	25.5±2.1 bc	510.3±94.4 ab	13.3 cd	48.8 b-d	2.7±0.9 a-c
10 ppm	70.0 b	2.2±0.1 a-c	25.8±2.9 bc	461.3±61.0 a-c	14.2 b-d	66.3 a-c	3.0±0.5 ab
20 ppm	62.4 bc	2.7±0.4 ab	33.4±4.1 b	704.7±103.8 a	10.7 cd	68.4 ab	2.7±0.6 a-c
30 ppm	50.0 cd	2.2±0.3 a-c	25.2±5.5 bc	405.9±138.3 b-d	9.3 d	50.7 b-d	1.7±0.8 a-e
AuNPs in the recovery medium							
0 ppm	46.0 cd	1.8±0.2 b-d	19.0±3.4 c	267.4±110.7 b-d	17.1 bc	19.3 de	1.0±0.8 c-e
10 ppm	40.0 de	2.6±0.2 ab	23.4±2.3 bc	362.4±61.2 b-d	24.4 a	32.0 b-e	0.7±0.4 de
20 ppm	27.0 ef	2.8±0.4 a	21.6±2.9 c	366.7±99.4 b-d	16.0 b-d	30.0 c-e	0.6±0.4 de
30 ppm	15.7 f	2.1±0.3 a-c	15.6±2.0 c	144.8±36.1 d	14.0 b-d	4.8 e	0.2±.02 e

AuNPs usually had no impact on the MSI (73.9–85.9%), except for those added into the recovery medium at the concentration of 30 ppm (decline to 55.8%). All LN-derived shoots were shorter and contained less chlorophyll and carotenoids than the untreated standard. Moreover, the application of AuNPs affected the enzymatic activity in *L. spectabilis*. Minor genetic variation was found in 8.6% of plants if AuNPs were added either into the preculture medium (at 10 and 20 ppm) or to the alginate matrix (at 30 ppm).

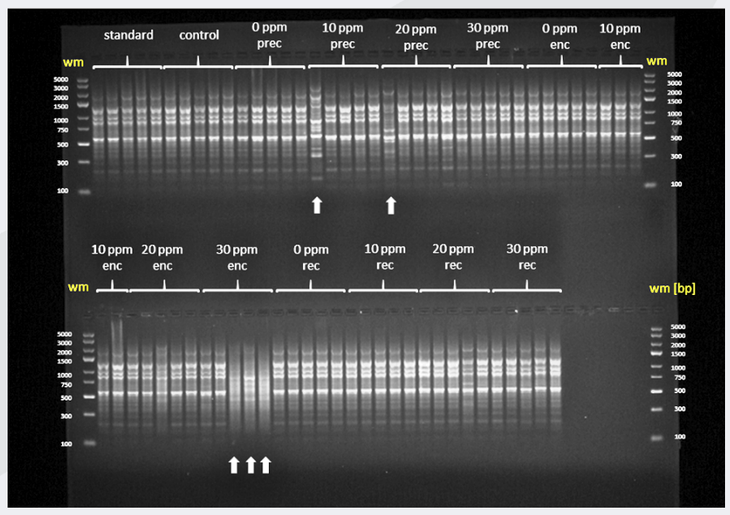

Fig. 3. Results of SCoT analysis. Arrows point to profiles that differ from the reference.

In **conclusion**, AuNPs added at a lower concentration (10 ppm) into the protective bead matrix can significantly improve the cryopreservation efficiency in *L. spectabilis* with no alternation in the DNA sequence.

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