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IMPROVEMENT OF *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. ENCAPSULATION-DEHYDRATION CRYOPRESERVATION PROTOCOL

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Abstract. To optimize the protocol, the shoot tips of *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. radiomutants have been cryopreserved using the encapsulation-dehydration technique, following different approaches. In the experiment the influence of abscisic acid – ABA (0; 10; 20 and 30 µM), sucrose concentration (0.09; 0.25 and 0.5 M) and temperature (5ºC or 22ºC) during preculture were tested. Furthermore two dehydration methods, gradual and one-step, were considered. Since the best results were reported with 10 µM ABA + 0.09 M sucrose at room temperature and gradual dehydration, only this combination was selected for the next desiccation-adjustment step. From the time of air desiccation under laminar air-flow applied (0; 1; 2; 3; 4 and 5 hours), 3 hours provided the best results. Due to the growth inhibition on the hormone-free medium, the addition of plant growth regulators during that phase was also tested, confirming the need to apply cytokinins into the medium.

Key words: mutant, ABA, sucrose, preculture, desiccation, regrowth

INTRODUCTION

Chrysanthemum × *grandiflorum* (Ramat.) Kitam. is the second biggest global floricultural crop following the rose [Teixeira da Silva and Fukai 2003]. The demand for new cultivars has been growing continuously every year. Mutation breeding is the most frequently applied method of achieving new cultivars of the species [Zalewska et al. 2011]. Every year hundreds of new cultivars (many of which, are unstable periclinal chimeras), replacing the current assortment, are produced. However, due to the change in consumer preferences, the cultivars which are out of fashion today may once again be attractive for potential consumers. Moreover, they are a great breeding material source; hence the protection and storage of valuable genetic resources are gaining importance. Today, cryopreservation; i.e. the storage of plant material at ultra-low temperature of

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liquid nitrogen (LN, -196ºC) is believed to be the most promising, cost-effective and safe diverse-biological-material storage method. Most modern cryopreserveation techniques based on vitrification (rapid transition of intracellular water to an amorphous glass state without ice crystallization), require the use of toxic compounds such as: dimethyl sulfoxide (DMSO) or ethylene glycol (EG), which can be especially harmful for mutant-unstable, slow-growing under *in vitro* conditions, chimeric cultivars and which can disturb their structure, leading to serious losses in the horticultural industry. The encapsulation-dehydration technique, thanks to the presence of a physical protection bead and a lack of toxic substances, seems more preferable and a higher genetic stability guarantee. However, the previous studies with *C. grandiflorum* encapsulation-dehydration were rather unsatisfactory resulting in a low survival, regrowth or regeneration through callus [Halmagyi et al. 2004, Martin and Gonzalez-Benito 2009, Osorio-Saenz et al. 2011a].

The aim of this study was to improve an easy, efficient and cost-effective *C. grandiflorum* radiomutants encapsulation-dehydration cryopreservation protocol, without the need of applying toxic and expensive cryoprotectants, by optimizing each major step of the technique, for the purpose of horticultural production, storage and breeding.

MATERIALS AND METHODS

To optimize the successive steps of the encapsulation-dehydration protocol, the experiment has been conducted, in four phases. To rationalize the expenditure of labor and materials, to every next step (preculture, osmotic dehydration, desiccation and recovery) only the combination of factors which provided the best results in the previous stage was selected.

Plant material. The biweekly (two weeks old) shoot tips of *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. 'Lady Salmon' and 'Lady Rosy' radiomutants, produced in the Laboratory of Biotechnology of the Department of Ornamental Plants and Vegetable Crops, 0.5–1.5 mm in length, with one or two leaf primordia, isolated with the use of microscalpels, surgical tweezers (to minimalize the injury) and a binocular microscope were used in the experiment.

The influence of abscisic acid. To stimulate the growth of shoot tips, the single-node explants of *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. 'Lady Salmon' with cut-off leaves, were cultured for one week on the modified MS [Murashige and Skoog 1962] medium with 0.09 M (standard 3% w/v) sucrose concentration, and additional 330 $mg \cdot dm^{-3}$ calcium chloride ($CaCl_2 \cdot 6H_2O$), 13.9 $mg \cdot dm^{-3}$ iron (II) sulphate ($FeSO_4 \cdot 7H_2O$) and 20.6 $mg \cdot dm^{-3}$ disodium ethylenediaminetetraacetic acid ($Na_2EDTA \cdot 2H_2O$) added, solidified with agar at the concentration of 8 $g \cdot dm^{-3}$, at pH 5.8 prior autoclaving in glass jars (10 explants per jar). After one week the explants were transferred onto the same medium, however, supplemented with 0; 10; 20 and 30 µM (0; 2.64; 5.28 and 7.92 $mg \cdot dm^{-3}$) of ABA – abscisic acid (40 explants per each concentration) for another 7 days.

All the explants were grown in the growth room at 24ºC ±2ºC, exposed to a 16-h photoperiod and photosynthetic photon flux density of 27.40 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

The isolated shoot tips have been incubated for 10 min in 3% sodium alginate (Na-alginate) based on the modified (as above) MS salts, without $CaCl_2$, supplemented with 0.09 M sucrose and then dipped into 0.1 M $CaCl_2$ for 30 min, forming calibrated beads (3 mm in diameter), after which the beads were rinsed three times with distilled sterile water to stop the alginate polymerization.

The beads were dehydrated for seven days gradually (4 steps) in 25ml of MS modified liquid media with increasing sucrose concentrations in 100 ml Erlenmeyer flasks at 130 rpm in the dark. The explants were transferred every 48 h to a fresh medium with the following carbohydrate concentrations: 0.3 M (48 h); 0.5 M (48 h); 0.7 M (48 h) and finally 0.9 M (24 h). Next the beads were desiccated for 3 h under sterile laminar air flow.

The explants were placed in 2.0 ml cryovials and immersed in LN (-196ºC). After one hour, the cryovials had been removed from the LN, thawed rapidly in water bath (38 ±1ºC for 3 min) and the beads were inoculated in Petri dishes on the modified MS semi-solid medium (4 $g \cdot dm^{-3}$ agar) with 0.09 M sucrose, and incubated in the growth room in the dark. After 48 hours the beads were transferred to a reduced lighting conditions (12.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 5 days. One week after thawing, the beads were transferred on a solid modified MS medium (10 beads per jar) supplemented with 0.09 M sucrose and 0.1 $mg \cdot dm^{-3}$ 6-benzylaminopurine (BA) and cultured under initial lighting conditions.

The influence of sucrose concentration, temperature during preculture and osmotic dehydration protocol. The single-node explants of 'Lady Rosy' chrysanthemum were cultured on the same modified MS medium, as described above, but supplemented with three different sucrose concentrations: 0.09 M (control; standard 3% w/v), 0.25 M (8.5% w/v), 0.5 M (17% w/v) sucrose (40 explants per each combination; 10 explants in a jar) and cultured in the same growth room. After seven days, the explants were transferred to the same media with an addition of 10 µM ABA for another one week.

Half of the explants from each sucrose concentration have been cultured in the growth room at 24ºC ±2ºC, under 16-h photoperiod and half in a refrigerator at 5ºC ±2ºC in the dark.

The shoot tips had been isolated and encapsulated as described above, but with Na-alginate supplemented with 0.09 M, 0.25 and 0.5 M sucrose (respectively to the conditions from the preculture phase). The vessels with explants which had been cultured at 5ºC were incubated at 0ºC (on ice) during the procedure.

The beads were dehydrated gradually (four or seven days) in the MS modified liquid medium in the dark with the following sucrose concentration: 0.3 M (24/48 h); 0.5 M (24/48 h); 0.7 M (24/48 h) and, finally, 0.9 M (24 h) at 130 rpm.

The survival of the encapsulated shoot tips from the control medium (0.09 M) precultured at 24ºC ±2ºC and subjected to a 4-days dehydration (without further desiccation and freezing) was measured after each transfer to a medium with a higher sucrose concentration, by inoculating the beads on the PGRs-free MS medium.

Additionally the explants from the control medium, incubated at room temperature were also osmotically dehydrated with one-step method (during which to lower the water content the beads were incubated only in a single sucrose solution) with

0.5 (45 h); 0.7 (18/45 h) or 0.9 (18 h) M carbohydrate concentration (20 explants per each treatment).

Next the beads were desiccated for 3 h, frozen in LN, thawed and transferred to a recovery medium (MS + 0.1 mg·dm^{-3} BA; 10 explants in a jar) as already described.

The influence of desiccation (physical dehydration) duration. The shoot tips of 'Lady Rosy' chrysanthemum from the control medium (0.09 M sucrose; supplemented with 10 µM ABA during the second week of preculture), incubated at room temperature, encapsulated and subjected to a gradual 4-days osmotic dehydration (0.3, 0.5, 0.7 and 0.9 M sucrose; 24 h for each concentration) were next desiccated for 0, 1, 2, 3, 4 or 5 h; 20 explants per each treatment), with or without subsequent LN treatment.

Recovery medium composition. For 'Lady Salmon' explants from the control medium (supplemented with 10 µM ABA during the second week of preculture), incubated at room temperature, encapsulated and subjected to a gradual 4-days dehydration followed by 3 h desiccation and LN treatment, several other recovery media, with: 0.1 mg·dm^{-3} BA; 0.1 mg·dm^{-3} kinetin (KIN); 0.1 mg·dm^{-3} (1-naphthaleneacetic acid) NAA; 0.1 BA mg·dm^{-3} + 0.05 mg·dm^{-3} NAA; 0.1 mg·dm^{-3} KIN + 0.05 NAA mg·dm^{-3} and hormone-free (40 explants per treatment), were tested.

Statistical analysis. The survival of the explants (% of shoot tips which survived freezing and remained green 14 days after thawing) and regrowth (% of explants which survived freezing and showed signs of further development 30 days after thawing) were estimated.

The results (completely randomized design) were statistically analyzed with the analysis of variance (ANOVA) and the comparisons of means were made with Tukey's Multiple Comparison Test ($p < 0.05$) using Statistica 10.0 and ANALWAR-5.2-FR tools.

RESULTS

The influence of abscisic acid. The presence and concentration of ABA was essential for the protocol efficiency (fig. 1), since over 2 times higher survival and an almost 1.7 higher regrowth were recorded with low (10 µM) concentration of the plant growth regulators (PGRs), in comparison to the hormone-free control. However, these parameters did not differ significantly, when comparing moderate (20 µM) or higher (30 µM) ABA concentrations with the control (42/39%; 22/20% and 40/30% survival/regrowth, respectively).

The influence of sucrose concentration, temperature during preculture and osmotic dehydration protocol. Sucrose concentration had a significant influence on the cryopreservation protocol efficiency (tab. 1). The highest survival and regrowth (64.5 and 58.2%, respectively) was observed with the lowest (0.09 M) sucrose concentration. There were no differences within these parameters with moderate (39.8/34.2%) and high (35.5/31.5%) carbohydrate concentrations.

As for the survival and regrowth, neither the temperature (5ºC or 22ºC) used during preculture (survival: 45.7 and 47.5%, respectively; regrowth: 41.0 and 41.2%, respectively) nor the time (4 or 7 days) of osmotic dehydration (survival: 45.8 and 47.3%,

respectively; regrowth: 43.5 and 39.2%, respectively) are acting independently, however, there were some significant interactions between the time of dehydration and temperature or sucrose concentration during preculture (tab. 1).

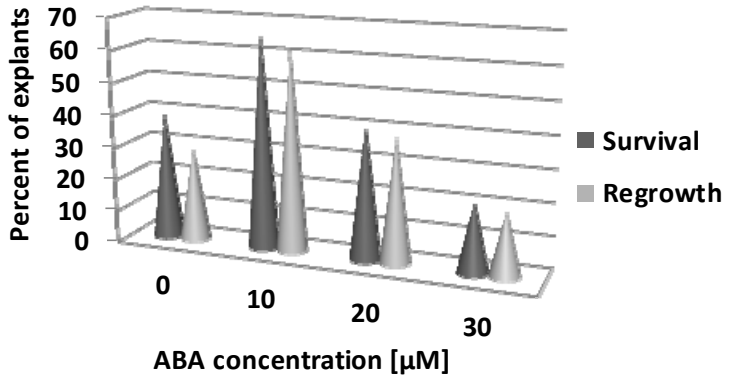

Fig. 1. Influence of ABA concentration on the 'Lady Salmon' explants survival and growth potential

With a 4-days dehydration, the lowest (0.09 M) sucrose concentration during preculture provided the best survival (55%; independently of the temperature), while there were no differences between the higher (0.25 and 0.5 M) concentrations (40 and 42%, respectively). The results were more diverse after a 7-days osmotic dehydration, leading to a significant survival decrease with growing sucrose levels (73.5, 39.5, 29%, respectively). The survival of the explants cultured on the MS + 0.09 M sucrose medium was significantly higher after a 7 days dehydration in comparison to the shorter path. The situation was quite the opposite with the highest (0.5 M) sucrose concentration tested. There was no difference between two times of dehydration with the medium (0.25 M) sucrose concentration.

As for the regrowth potential, there were no differences between following sucrose concentrations after a 4-days dehydration (50, 35.8 and 42%, respectively). However, after the longer (7-day) path there was a significantly higher recovery observed after applying the lowest sucrose concentration (66.5%) compared to higher levels (30 and 21%, respectively). The regrowth of the explants cultured on the medium with the highest sucrose concentration was significantly higher after a 4 days dehydration in comparison to the longer path. There were no interactions between other sucrose concentrations and the two dehydration periods.

The influence of temperature was dependent by the time of dehydration. It was non-significant in most of the cases, except for the 7-day dehydration preceded by preculture on the MS medium + 0.09 M sucrose when a significantly higher survival was observed after incubation at 5ºC compared to 22ºC, and to 5ºC followed by the 4-day dehydration (tab. 1).

Table 1. Survival and regrowth of the frozen 'Lady Rosy' explants from different preculture conditions

Sucrose concentration (M)	4-day osmotic dehydration				7-day osmotic dehydration			
	22ºC		5ºC		22ºC		5ºC	
	Survival (%)	Regrowth (%)	Survival (%)	Regrowth (%)	Survival (%)	Regrowth (%)	Survival (%)	Regrowth (%)
0.09	56aB*	50aA	**55aB**	50aA	**67aA**	63aA	**80aA**	70aA
0.25	44bA	44aA	36bA	33aA	38bA	25bA	41bA	35bA
0.5	44bA	44aA	40bB	40aA	25cB	20bB	33bB	22bB

* means marked with the same letter do not differ significantly at $\alpha = 0.05$; lower-case letters refer to sucrose concentration in the same conditions; upper-case letters refer to the dehydration time in the same conditions; means marked in bold characters differ significantly in terms of temperature conditions

The highest recovery was observed after a 7-days dehydration, preceded by preculture on a medium supplemented with 0.09 M sucrose, while the lowest, with the same dehydration time, but with 0.5 M sucrose application, independently of the temperature.

There was no difference in survival observed between 14 and 30 days after thawing, except for the shoot tips precultured on the control medium (with 0.09 M sucrose during preculture) at 5ºC and subjected to a 7-days dehydration.

The influence of the following steps of osmotic dehydration (0.3, 0.5, 0.7, 0.9 M sucrose) on the explants survival is shown in Figure 2. The apparent survival decrease (90% without dehydration and 79% after the final dehydration step with 0.9 M sucrose) was non-significant.

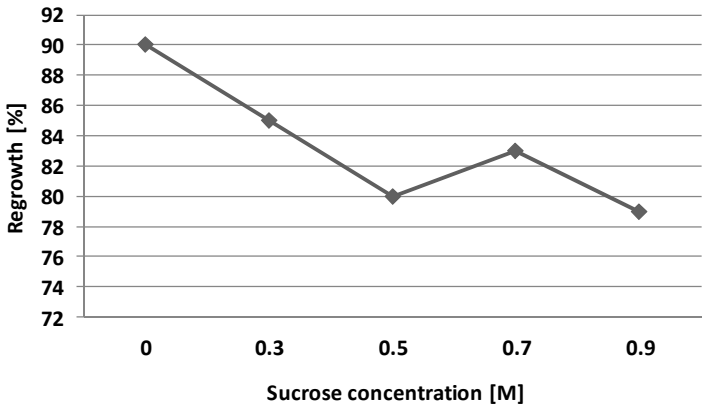

Fig. 2. 'Lady Rosy' explants survival during osmotic dehydration

As for one-step dehydration, 2 days after thawing, the survival reached from 14% to even 67% after previous 45 or 18-hour bead incubation with 0.75 M sucrose, respectively. This was however non-significant, since all the explants after one-step dehydration (0.5 (45 h); 0.7 (18/45 h) and 0.9 (18 h) M sucrose) turned brown and died two weeks after thawing.

The influence of desiccation (physical dehydration) duration. Air desiccation is one of the most critical phases (fig. 3). A prolonged drying time (4 and 5 hours) leads to significant survival decrease (50–70%), even without the treatment with LN, whereas up to 3 hours, the survival does not fall below 80% (non-significant decrease compared to non-desiccated control).

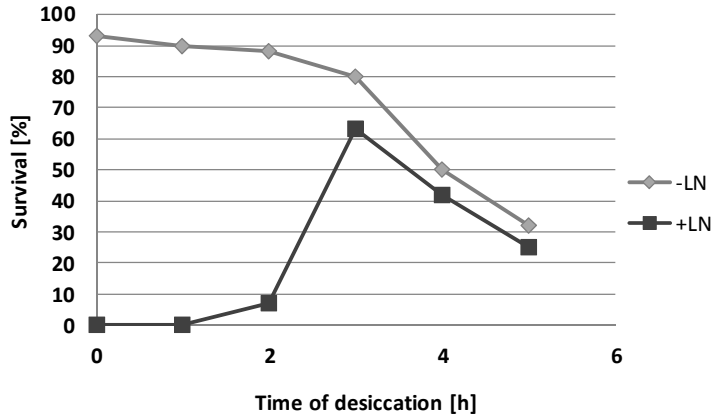

Fig. 3. Influence of desiccation time on the 'Lady Rosy' explants survival

The experiment with LN treatment proved that a 3-hour desiccation under laminar-air flow seems optimal, resulting in over 60% regrowth. There are no significant differences between 3 and 4-hour drying, whereas any longer time of exposition leads to a decrease in the value of this parameter. The air drying shorter than 3 hours does not guarantee any significant survival (or very low; below 10% as for 2 h).

Recovery medium composition. It was observed very often that even two days after thawing, all explants remained green, regardless of the combination applied, however a significantly lower and more diverse survival rates after 14 days were reported.

The lowest recovery was observed on the PGRs-free medium and in the presence of NAA auxin alone (16–20%; non-significant differences; fig. 4). The shoot tips inoculated on the hormone-free regeneration medium, grew slower than the unfrozen control which produced green healthy microshoots of a typical morphology. Some of the explants demonstrated the first signs of growth (primordial development) a few days after thawing, however, they died later.

In the presence of cytokinins significantly higher regrowth rates (39–47%) were observed, however, there were no differences noticed among different media with Kin or BA. The shoot tips inoculated on the MS medium supplemented with 0.1 $mg \cdot dm^{-3}$ BA

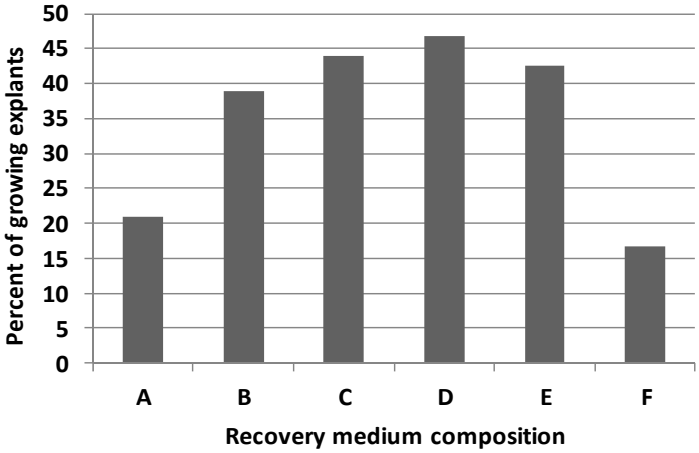

Fig. 4. Influence of recovery medium composition time on the 'Lady Salmon' explants survival: A – 0.1 mg·dm^{-3} NAA; B – 0.1 mg·dm^{-3} Kin; C – 0.1 mg·dm^{-3} BA; D – 0.1 mg·dm^{-3} Kin + 0.1 mg·dm^{-3} NAA; E – 0.1 mg·dm^{-3} BA + 0.1 mg·dm^{-3} NAA; F – PGRs-free control

or KIN produced shoots, although the growth of the explants from the medium with kinetin (which produced only single shoots) was slower in comparison to the medium with BA (producing both single and multiple shoots). Still the microshoots from the BA-supplemented medium had short internodes than the control. Also the presence of callus was observed after using this cytokinin. After applying the combination of auxins and cytokinins a somewhat faster plantlet development (growth and breaking of capsules by the microshoots) was observed.

DISCUSSION

Plant crypreservation has a relatively short history. The first developed cryopreservation manuals were based on the controlled-rate freezing. Despite this technique being successful with some *Chrysanthemum* species (47–91% regrowth for *C. grandiflorum*) [Fukai 1990, Fukai et al. 1991], it is very expensive, for it requires a special cooling apparatus. Vitrification-based methods are more preferable than two-step freezing. Among them, encapsulation-dehydration proved to guarantee the fastest recovery of the explants [Mikuła et al. 2005], which is especially important with slow-growing mutant cultivars, very popular among chrysanthemums. The experience shows that the encapsulation-dehydration technique provides better protection than plant vitrification solution (PVS – a concentrated mixture of cryoprotectants) or sucrose pretreatment alone [Mikuła et al. 2005], which is also important with chimeric forms. Furthermore, in the vitrification protocols, small explants manipulation is necessary, which makes it technically difficult. Closing the explants in a protective capsule makes it much easier. Encapsulation has already been used for *Chrysanthemum* artificial seeds production [Pinker and Abdel-Rahman 2005].

Over the years, the shoot tips of numerous *Chrysanthemum* species have been cryopreserved following different techniques [Fukai 1990, Fukai et al. 1991, Halmagyi et al. 2004, Martin and Gonzalez-Benito 2009]. The results reported in the experiment (70% regeneration of the explants in the best conditions) are very satisfying since Sakai et al. [2000] and Halmagyi et al. [2004] recorded a 20–45% shoot recovery. Similarly as described by Halmagyi et al. [2004], not all the apices showing survival developed into shoots. Although Popova et al. [2010] achieved better results with droplet-vitrification technique (91% recovery with 'Peak' cultivar), the experiments performed by Fukai et al. [1994] proved that the chimeric structure of the chrysanthemum apical dome is affected by the presence of PVS, the presence of which is not necessary in the encapsulation-dehydration technique.

Preculture is sometimes the only condition required to protect the plant from a destructive influence of lower temperatures, as demonstrated by Hitmi et al. [2000] who, by subjecting the shoot tips of *Chrysanthemum cinarariefolium* to a 3-day preculture on the MS medium, containing 0.55 M sucrose + 4 μM ABA, before immersion in LN, reported a 76% recovery. Also in our experiment, the presence of ABA was essential resulting in an over 2 times higher survival in comparison to the PGRs-free control. Similarly, Sakai and Engelmann [2007] reported the positive role of cold acclimation (at 5–10ºC) on the cryopreservation efficiency. Although this was confirmed with *Chrysanthemum* × *grandiflorum* 'Shuhounochikara' by Sakai et al. [2000], no such influence was observed with 'Lady Rosy'.

For developing a cryopreservation protocol, it is necessary to take into account the natural properties of the species: its desiccation and freeze tolerance; a quantitative trait under polygenic control [Deng et al. 2011]. As already mentioned, most of the chrysanthemum cultivars are not cold hardy [Deng et al. 2011]. Our experiment demonstrated that 'Lady Rosy' is also susceptible to prolonged dehydration, which can be why fewer explants survived freezing after preculture on the media with higher sucrose concentrations and which can account for a low survival after 4- and 5-hour air desiccation (even without LN treatment). Also Hosoki [1989] observed the highest (55%) *C. grandiflorum* shoot tips survival on the media with the lowest (2%) sucrose concentration and its decrease with higher (4 and 6%) carbohydrate levels. Bachiri et al. [1995] explained that the permeability for sucrose of the plasmalemma depends on the biological material source. An excessively strong dehydration may cause the cell death as a result of progressing plasmolysis or osmotic shock [Wilkinson et al. 2003]. The highest survival (64.5%) and regrowth (58.2%) of the explants precultured on the medium with the lowest sucrose concentration is especially significant since Martin et al. [2011] proved that treatment with high sucrose concentration is the main cause of genetic instability in cryopreserved *C. grandiflorum* apices. In the future, *C. grandiflorum* desiccation tolerance could be induced by the addition of trehalose instead of sucrose during preculture [Osorio-Saenz et al. 2011b]. The addition of proline could also enhance the species tolerance to cold and desiccation stress, as noticed with related *Ajania przewalskii* [Deng et al. 2011].

In general, dehydration decreases the survival and growth rate of the unfrozen materials. It is, however, necessary when applying LN, which was also proven in the experiment conducted. Fukai et al. [1990] suggested that chrysanthemums showed a low

dehydration rate, which agrees with our experiment, since there was no critical level of sucrose used during osmotic dehydration, which would significantly influence the shoot tips survival (80–90%).

The experiment has proven that with 'Lady Rosy' chrysanthemum a gradual dehydration is the only alternative, since even though relatively high survival after two days of cryopreservation with one-step dehydration (14–67% after 45 or 18 hour-incubation with 0.75 M sucrose), there was no survival observed two weeks after thawing. Similar results were obtained with *Rosa* 'New Dawn' axillary buds and *Phlebodium aureum* gametophyte [Pawłowska 2008]. Halmagyi et al. [2004], though, reported satisfactory results with *C. grandiflorum* 'Escort' shoot tips after a 4-hour incubation on the MS medium with 0.75 M sucrose, however, supplemented also with PGRs (1 mg·dm^{-3} BA, 0.1 mg·dm^{-3} NAA).

The optimal desiccation time (3 hours) observed in our experiment with 'Lady Rosy' is shorter in comparison to 'Pasodoble' chrysanthemum, which showed a similar 64% survival, but after 5 hours drying [Martin and Gonzalez-Benito 2005]. This emphasizes the sensitivity of 'Lady Rosy' to dehydration. A similar regrowth after a 4-hour desiccation (45%) was observed with *C. grandiflorum* 'Escort' [Halmagyi et al. 2004], although it was higher (45%) after 5 hours, as compared to 'Lady Rosy' (25%). With 'Shuhounochikara' chrysanthemum, 9 hours of desiccation resulted in a very low, 20%, shoot recovery, whereas a 3-hour desiccation preceded with a 1-hour preculture with 2.0 M glycerol + 0.4 M sucrose resulted in a 85% apices regrowth [Sakai et al. 2000].

The requirements towards the growth medium are cultivar-dependent. Chrysanthemums proved to be especially diverse in terms of requirements [Annadana et al. 2000]. PGRs played a key role in the development of microshoots under *in vitro* cultures conditions. This was also proven in the present experiment since the application of a hormone-free medium did not guarantee the growth of the thawed explants (16%), however it was sufficient for the unfrozen ones (80–90%). The presence of cytokinins was crucial for the development of the shoots, which was also noticed with *C. grandiflorum* 'Otomezakura' [Hosoki 1989]. Annadana et al. [2000] report on the difference in the regeneration efficiency depending on the cytokinin source. This was also found in our experiment, since BA stimulated a faster regeneration (often through callus phase) of multiple shoots. Kinetin on the other hand, stimulated only direct regrowth of single shoots. Unlike unfrozen shoot tips [Waseem et al. 2011], the presence of NAA alone was not sufficient to stimulate the shoot tips development (20%). However, the combination of BA or KIN (0.1 mg·dm^{-3}) and NAA (at a two fold lower concentration), stimulated a faster response of the explants (growth and breaking the capsule).

CONCLUSION

1. The presence of 10 μM ABA in the preculture medium is profitable, however in higher concentrations, it decreases the regrowth potential of the explants.

2. Low sucrose concentration (0.09 M), gradual dehydration and 3-hours desiccation are optimal for cryopreservation of 'Lady Salmon' and 'Lady Rosy' chrysanthemums.

3. The presence of cytokinins in the growth medium is essential for the regrowth of the thawed explants. Kinetin is more preferable than BA, since it prevents from the regeneration of multiple shoots of short internodes.

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OPTYMALIZACJA PROCEDURY KRIOPREZERWACJI CHRYZANTEMY WIELKOKWIATOWEJ (*Chrysanthemum* × *grandiflorum* (Ramat.) Kitam.) METODĄ KAPSUŁKOWANIA-DEHYDRATACJI

Streszczenie. W celu optymalizacji procedury pąki wierzchołkowe radiomutantów chryzantemy wielkokwiatowej pochodzące z różnych warunków kultury *in vitro* poddane zostały krioprezerwacji techniką kapsułkowania-dehydratacji. W trakcie doświadczenia zbadano wpływ kwasu abscysynowego – ABA (0; 10; 20 i 30 μM), stężenia sacharozy (0,09; 0,25 i 0,5 M) oraz temperatury (5ºC lub 22ºC) w trakcie prekultury. Uwzględnione zostały dwie metody dehydratacji (stopniowa i jednoetapowa). Ponieważ najlepsze wyniki uzyskano na pożywce zawierającej 10 μM ABA + 0,09 M sacharozy w temperaturze pokojowej i po zastosowaniu stopniowej dehydratacji, tylko tę kombinację wykorzystano w kolejnym etapie – optymalizacji czasu desykacji. Spośród zastosowanych okresów suszenia (0, 1, 2, 3, 4 i 5 godzin), suszenie przez 3 godziny zapewniło najlepsze wyniki. W związku z zahamowaniem wzrostu roślin na pożywce pozbawionej regulatorów wzrostu określono wpływ auksyn i cytokinin na ten proces, potwierdzając konieczność stosowania tych ostatnich.

Słowa kluczowe: mutant, ABA, sacharoza, prekultura, suszenie, wzrost

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