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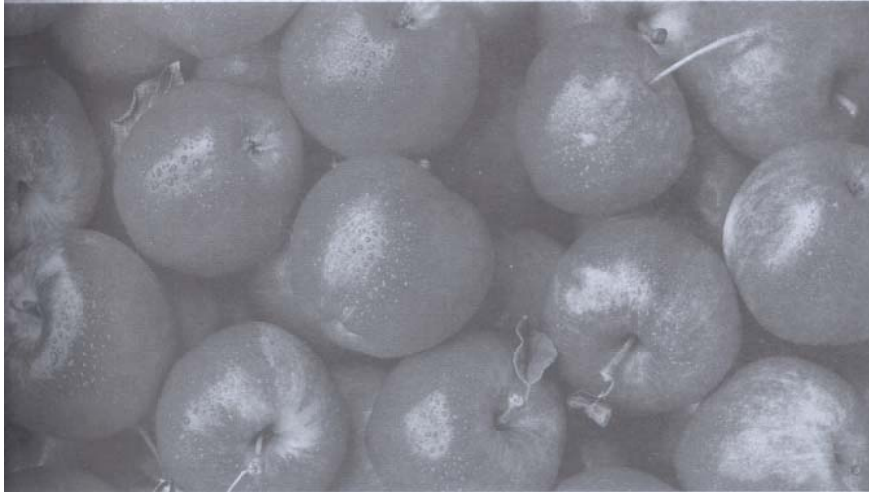
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quences of different N fertilization regimes applied to poinsettia stock plants and cold dark storage of cuttings on N accumulation, carbohydrate distribution, and rooting competence of cuttings were investigated. Increasing time of stock plant cultivation with graduated N fertilisation produced cuttings with N contents, ranging from 19 to 51 mg-N·g⁻¹ dry mass (DM). High N accumulations resulted in low carbohydrate concentrations in cuttings, and subsequent storage decreased carbohydrate concentrations further, particularly in stems. Reduced rooting of stored cuttings correlated with lower sucrose contents in leaves at a particular harvest date. However, despite the lower carbohydrate levels, root numbers and lengths correlated positively with N concentrations. These relationships remained stable in unstored and stored cuttings, even when overall rooting intensity was reduced under lower natural light during autumn. Multivariate regressions highlighted these relationships and explained up to 79% of rooting variances while nitrogen content and daily light integral during rooting turned out to be predominant factors. N content in cuttings is considered to be the most dominant factor determining the rooting capacity of poinsettia when rooting occurs under sufficient light, as is commonly available during propagation. Nitrogen content in poinsettia cuttings should surpass a threshold of 40 mg-N·g⁻¹ DM to maximize their rooting potential.

S09.350

Cryopreservation of Rosa (*Rosa canina* L.) Apical Meristems by Droplet Vitrification Method

Pawlowska, B.

UNIVERSITY OF AGRICULTURE IN KRAKOW, DEPARTMENT OF ORNAMENTAL PLANTS, AL. 29 LISTOPADA 54, 31-425 KRAKOW, POLAND

The most popular and frequently occurred species of roses in Poland is *Rosa canina*, which is also widely used in ornamental horticulture. Important strategy of the genes resources is ex situ protection, polegająca na przeniesieniu pojedynczych osobników lub ich grup from the nature to artificial conditions, to multiplication them or to reserve genus pool. It is made by create gene banks and *in vitro* collection and additionally preserved in liquid nitrogen. Moreover application of cryopreservation (-196 °C) cultures reduces somaclonal variability in *in vitro*. In the present studies, the protocol of preservation of shoot meristems of wild rose (*Rosa canina* L.) in liquid nitrogen by the droplet vitrification method was developed. Apical meristems of about 1-2 mm were collected from plants propagated *in vitro* on the MS mineral medium (1962), supplemented with 10 μM BA, 0.3 μM GA3 and 0.06 M sucrose. Moreover, some plants were cultured on the medium containing higher sucrose level - 0.25 M for 4 weeks. After excised all meristems for cryopreservation were precultured on the liquid medium containing higher sucrose level 0.1 – 1 M for 24 hours. Then explants placed on sterilized aluminium foil strips were dehydrated with concentration PVS2 cryoprotective solution (4 μl) for 10 - 30 minutes at room temperature and plunge to liquid nitrogen. Rewarming of samples was performed in liquid medium and meristems were placed for regeneration medium with 0.5% of agar. The obtained results indicate that survival rate of apical meristems after freezing in liquid nitrogen depended on concentration of sucrose in regeneration medium. Also preculture liquid medium and time operation of PVS2 were effected on regeneration after freezing.

S09.351

Observation of Structural Characteristics of Petal Tissue in Preserved Flower of Rose 'Heaven'

Yoo, E. H.; Do, G. R.; Kim, K. J.; Jeong, S. J.; Han, S. W.; Jeong, M. I.; Lee, D. W.; Song, J. S.

NATIONAL INSTITUTE OF HORTICULTURAL & HERBAL SCIENCE, 540-41, TAP-DONG, 441-440, SUWON, REPUBLIC OF KOREA

The floral decorations business demands a steady supply of marketable processed plant materials such as dried flowers as well fresh flowers. To satisfy this demand, the following properties are required in plant decorations: attractive shapes, colors, fragrance, flexibility, consistent appearance, profitability, and consumer preference. A preserved flower is a type of processed flower that is dehydrated by

replacing the moisture of its tissues with one of various preservative solutions. Preserved flowers are unique in that unlike fresh flowers, they have a long vase life of several years and yet they are not brittle, unlike dried flowers. In this research, the differences in the ultra-structural characteristics of petal tissues of fresh, dried, and preserved roses were investigated. In the process of making a preserved flower, after the petal tissues were dehydrated for 24 hours, they became firm and still maintained the original shape of their epidermis layer, unlike the tissues of an air-dried flower. Thus, the flower tissues that were treated with a preservative preserve their tight texture. Due to the stable firmness of the petal tissues of the preserved flower, their structural characteristics could be observed via SEM without any other pre-treatment. Because the tissues of fresh flowers were so weak, however, they easily broke in a vacuum for SEM observation. In this research, the main differences in the morphological characteristics of fresh flowers, dried flowers, and preserved flowers were determined. No differences were found, however, among the preserved flowers, which were made using different composition ratios of preservative solutions.

S09.352

Suspended Callus Culture of the Endangered Orchid *Dendrobium loddigesii* Rolf.

Yi, Y.¹; Xu, X.¹; Zhang, Y.¹; Yang, L.¹; Zhang, D.²

¹SCHOOL OF LIFE SCIENCES, GUIZHOU NORMAL UNIVERSITY, 550001, GUIYANG, GUIZHOU, CHINA

²UNIVERSITY OF MAINE, ORONO, MAINE, UNITED STATES

Dendrobium loddigesii Rolf is famous for its specific pink flowers in China. As both ornamental and traditional medicine plant, the species in wild is becoming more and more exiguous because of small distribution, low seed germination and excess utilization. In order to protect and avail wild resource of *Dendrobium loddigesii* reasonably, suspended callus culture of this species in 250mL of Erlenmeyer flask was studied and growth kinetics was analyzed. Different basic media (MS, 1/2MS, B5 and WS), plant growth regulators (2,4-D, NAA, IAA and IBA), carbon resources (glucose, sucrose) and other conditions. The results showed that the optimal medium for suspension culture is MS + 2,4-D 0.1 mg/L + 3% sugar with 100rpm of rotation, temperature at 23 ± 2 °C, photoperiod at 12h/12h, photo radiation at 1500-2000 lx, and the medium changed every 28-31 days.

S09.353

Propagation of *Pancreatium maritimum* for Use as Ornamental or Garden Plant

Nesi, B.; Trinchello, D.; Lazzereschi, S.; Grassotti, A.

CRA-VIV, VIA DEI FIORI, 8 - 51012 PESCIA, PISTOIA TUSCANY, ITALY

Pancreatium maritimum (*Amarillidaceae* Family), originally distributed along the coasts of the Mediterranean sea, owes its ornamental value to the white floral scapes pleasantly scented. The development of *in vitro* and *in vivo* procedures for its massive propagation is necessary for the reintroduction in its original sandy sites and for its exploitation as ornamental plant. *In vitro* experiment, started from portions of the bulb (twin and tri-scales). The bulbs were sterilized and the explants were grown on culture media containing 4 different concentration of Benzyladenine (BA) (0, 1, 4.4, 10 and 22.2 mg/L) at 22 °C. All the explants were previously kept in the dark, and for the remaining period, were transferred half in the dark and the half under a photoperiod of 12 hours. After 60 days of *in vitro* culture, a bulb production from portions of scales was obtained with a percentage viability of 22%. After 180 days the percentage of forming bulbs explants was evaluated: for the twin-scales, the best BA concentration was of 1 and 4.4 mg/L, with a percentage of forming bulbs explants of 19%. For the tri-scales, the best concentration of BA, that allowed to obtain the highest generation of bulbs (25%), was of 1 mg/L. As regard *in vivo* activity, the objective was the evaluation of seeds germination in response to light (dark or 12 h photoperiod), temperature (4 °C, 20 °C and 30 °C) and gibberellin (0 - 2,9 - 29 mM). The seeds kept in the dark always showed higher and earlier germination rate. Furthermore, low temperature inhibited germination, while temperatures of 20 °C and 30 °C induced a higher germination.

Suspended Callus Culture of the Endangered Orchid *Dendrobium loddigesii* Rolfe

Yin YI¹, Xiaorong XU¹, Yubin ZHANG¹, Lichang YANG¹, and Donglin ZHANG²

¹School of Life Science, Guizhou Normal University, Guiyang, Guizhou 550001, China

²Plant, Soil and Environmental Sciences, University of Maine, Orono, ME 04469, USA

Introduction

Dendrobium loddigesii Rolfe is famous for its specific light pink flowers in China. As an ornamental and traditional medicinal plant, the species in wild is becoming endangered because of its narrow distribution, low rate of natural regeneration and excessive collection. In order to protect and utilize wild resource of *D. loddigesii* properly, suspended callus culture of this species was investigated and growth pattern was analyzed.



Figure 1: The flower of *D. loddigesii* Rolfe



Figure 2: The suspension culture

Material & Methods

The fresh plants of *D. loddigesii* was supplied by the key laboratory of molecular biology at Guizhou Normal University.

The explants from stems or other parts of *D. loddigesii* were inoculated in MS, 1/2MS, B5 and WS media, respectively. Various concentrations of NAA, 6-BA and 2,4-D were added. The cultural conditions were $25 \pm 2^\circ\text{C}$, 12h/12h(day/night), 1500~2000lux illumination and rotation for 120rpm. The calluses were counted after the explants had been grown in the media for 60 days.

The induced calluses were inoculated in the 100mL of conical flask containing 50mL of liquid MS and the conical flasks were weighed before and after inoculation, respectively. Some parameters such as callus weight, water content and propagation rate were obtained at the following conditions: 0, 0~500, 500~1000, 1000~1500, 1500~2000 lux of illumination, 12h/12h(day/night), 60rpm, 80rpm, 100rpm, 120rpm of rotation rate, and pH at 5.4~5.8, 5.8~6.2, 6.2~6.7 or 6.7~7.0.

Results & Discussion

Plant hormones affected callus inductions. Among them, 2,4-D had the best result (Table 1).

The results showed that the best medium for callus induction was the 1/2MS+0.1-2.0mg/L 2,4-D+3% sugar (Tab.1). Rotation rate affected suspension culture propagation of the callus of *D. loddigesii*. The results shown that 100rpm had the better callus growth (Table 2).

Light intensity affected suspension culture propagation of the callus of *D. loddigesii*. Among them, 500~1000lux of illumination was better one (Table 3).

The pH affected suspension culture propagation of the callus of *D. loddigesii*. The result indicated that the proper pH should be from 5.8 to 6.2 (data not presented).

Conclusion

In summary, we established the suspension culture condition for *D. loddigesii* Rolfe. The best cultural condition was liquid 1/2MS containing 0.1mg/L 2,4-D and 3% sugar, pH5.8-6.2, 100rpm of rotation rate, 12h/12h(day/night), 500~1000lux of illumination, and culture period of 60 days.

Table 1: Effect of plant hormone concentrations on callus induction of *D. loddigesii* Rolfe.

Medium (mg · L ⁻¹)	Callus	Shoot
MS+2,4-D(0.1~2.0)	+	-
MS+2,4-D(0.1~2.0)+NAA(0.1~2.0)	-	-
MS+2,4-D(0.1~2.0)+6-BA(0.5~2.0)	+	+
MS+NAA(0.5~2.0)	++	+
MS+NAA(0.1~2.0)+6-BA(0.5~2.0)	-	++
MS+2,4-D(0.1~2.0)+NAA(0.1~2.0)+6-BA(0.5~2.0)	-	++
1/2MS+2,4-D (0.1~2.0)	+++	-
1/2MS+2,4-D (0.1~2.0) +NAA (0.1~2.0)	-	-
1/2MS+2,4-D(0.1~2.0)+6-BA(0.5~2.0)	+	+
1/2MS+NAA(0.5~2.0)	+	+
1/2MS+NAA(0.1~2.0)+6-BA(0.5~2.0)	-	++
1/2MS+2,4-D(0.1~2.0)+NAA(0.1~2.0)+6-BA(0.5~2.0)	-	++

Note: - indicates no callus induced or the callus died, + indicates the induced callus (also in Table 2)

Table 2: Effect of rotation rates on callus induction of *D. loddigesii* Rolfe.

Rotation (rpm)	1/2MS Callus propagation	1/2MS+2,4-D Callus propagation
60	+	+
80	+	++
100	+	+++
120	+	++

Table 3: Effect of light intensity on callus induction of *D. loddigesii* Rolfe.

Light intensity (lux)	Callus growth
0	Ivory-white, big group
0~500	Buff, big group
500~1000	Yellow-green, big group
1000~1500	Yellow-green, big group
1500~2000	Browning