In Vitro Medium-Term Conservation of Several Carnation Accessions through Nutrient Modification

Kurniawan Budiarto*, Budi Marwoto, and Rudy Soehendi

Indonesian Ornamental Crops Research Institute (IOCRI), *Jl. Raya Ciherang, Segunung Pacet Cianjur 43253, West Java, Indonesia*
*Phone (62-263) 512607; Facs. (62-263) 514138; *E-mail: bud1arto@yahoo.com*

Submitted: 10 Januari 2013; Accepted: 18 April 2013

ABSTRACT

Modification of osmotic pressure of *in vitro* medium-term can be applied to conserve carnation accessions. This successful method inspired the use of nutrient modification technique to have the same function as far as these concerned. The research was then, conducted to evaluate *in vitro* media for preserving several carnation accessions in low temperature conditions. The research was carried out at Indonesian Ornamental Crops Research Institute (IOCRI) from January 2007 to March 2008. A randomized completely block design with 25 replications was used to accomplish the combination of two factors. The first factor was six commercial carnation accessions, namely cv. Light Pink Candy, Aicardy, Torpedo, Malaga, President, and Rendezvouz. While the second factor dealt with four formulations of conservation media i.e. ½ MS + DMSO 3% + 4% sucrose, modified hyponex + 4% sucrose, modified hyponex (no sucrose) and ½ MS + 4% sucrose (control). The results showed that medium term *in vitro* conservation for carnation were successfully conducted up to 10 months using in ½ MS + DMSO 3% + 4% sucrose and modified hyponex + 4% sucrose without any significant differences and decreases in viability when transferred in to shoot induction media. In the absent of sucrose, however, the plantlet survivals were only up to 8 months. No significant variation on viability and number of survival plantlet was observed among the carnation accessions in all media tested.

Keywords: *Dianthus carryophyllus*, accessions, medium-term conservation, *in vitro* media, low temperature.

ABSTRAK

Konservasi Jangka Menengah *In Vitro* Beberapa Aksesi Anyelir melalui Modifikasi Nutrien. Kurniawan Budiarto, Budi Marwoto, dan Rudy Soehendi. Modifikasi tekanan osmotik dapat digunakan untuk konservasi *in vitro* anyelir. Hal ini menginspirasi pengembangan teknik konservasi melalui metode modifikasi media. Metode modifikasi nutrien pada media konservasi diharapkan menjadi alternatif teknik konservasi *in vitro* jangka menengah pada anyelir. Penelitian ini bertujuan mendapatkan teknik konservasi *in vitro* jangka menengah beberapa aksesi anyelir melalui modifikasi nutrien media dan penyimpanan pada suhu 4^{o}C. Penelitian dilakukan di Balai Penelitian Tanaman Hias dari Januari 2007 hingga Maret 2008 dengan menggunakan rancangan acak kelompok (RAK) pola faktorial dengan 25 ulangan. Faktor percobaan meliputi 6 kultivar anyelir, yaitu cv. Light Pink Candy, Aicardy, Torpedo, Malaga, President, dan Rendezvouz dan 4 formulasi media konservasi, yaitu ½ MS + 3% DMSO + 4% sukrosa; hyponex modifikasi + 4% sukrosa; hyponex modifikasi tanpa sukrosa; dan ½ MS + 4% sukrosa sebagai kontrol. Hasil penelitian menunjukkan bahwa konservasi jangka menengah hingga 10 bulan pada ½ MS + 3% DMSO + 4% sukrosa dan hyponex modifikasi + 4% sukrosa dapat dilakukan terhadap anyelir tanpa penurunan signifikan pada viabilitas planlet di media induksi tunas. Sedangkan pada media tanpa sukrosa, yaitu hyponex modifikasi, ketahanan planlet hidup hanya mencapai 8 bulan. Tidak terdapat perbedaan jumlah planlet hidup dan viabilitas planlet yang signifikan antar aksesi anyelir yang dicoba pada semua media konservasi *in vitro*.

Kata kunci: *Dianthus carryophyllus*, askesi, konservasi jangka menengah, media *in vitro*, temperatur rendah.

INTRODUCTION

Carnation (*Dianthus carryophyllus*) is one of the most popular ornamentals in the world. The species is originated from southern Russia to Alpine Greece and the Auvergne mountains of France. The *Dianthus* species are adapted to the cooler Alpine regions of Europe and Asia, and are also found in Mediterranean coastal regions and up to this moment over 300 *Dianthus* species have been identified (Jurgens *et al*., 2003a). In Indonesia, the plant has been cultivated since long time ago as a mean of mainly ornamental and well adapted in highlands (Budiarto and Nasihin, 2007).

Traditional techniques for carnation breeding were developed before the discovery of inheritance principles by Mendel (Fukai and Tanaka, 2004). Variation in flower colors and shapes, leaves, flowering responses and pest and disease

resistances on the existing commercial cultivars reflected the complex combination of parentages and genetic constructions produced from breeding activities from the past up to this moment. In the absence of self-pollination, continuous hybridization has inadvertently resulted in highly heterozygous carnation cultivars for mono-ideotype (Jurgens *et al*., 2003b). The desirable character was improved, while some unpreferred characters were systematically swept out. These dynamic trends have also hastened the genetic erosion on such plant (Halmagyi and Deliu, 2007).

Preserving genetic diversity was considered a great deal of emphasis for the future breeding to important characters. Native from temperate region and limited life span in intact conditions in tropic area have made the maintenance of active growth plant in base collection at *in vivo* condition would be very laborious, expensive and risky from associated field such as pathogens, pests, climatic perturbation and human error (Diettrich *et al*., 1985).

In vitro conservation was considered to be one promising tools in preserving the collection and reduced the limitation of in vivo conservation. This conservation method was usually conducted using cell growth inhibitor and protectant. Previous study by Budiarto (2008) reported that medium-term *in vitro* conservation of carnation could be conducted through osmotic pressure method using DMSO (*Dymethil sulfoxide*). With these applications, the plantlets could be stored in low temperature (4^{o}C) up to 10 months without significant decrease of plantlet survival rates and viability after transferred to induction medium. In some accessions, however, various degree of phytotoxicity was observed in the media containing more than 3% DMSO.

Nutrient modification was reported to have similar function with osmotic pressure as growth inhibitor *in vitro* conservation. These methods have also been successfully applied for cryopreservation in some plants, such as citrus, cassava, and potato (Gonzalez-Arnao *et al.,* 2008). The hyponex based medium could be used in this case. With several particular modifications in the composition and/or additional defined elements, this medium has been used in germinating and preserving the seed of orchid plants (Nishimura, 1982). Considering the possibility of the use of nutrient modification medium, then *in vitro* conservation of several carnation accessions was studied. In this paper, medium-term *in vitro* conservation using modified hyponex medium during low temperature (4^{o}C) storage was investigated and described.

MATERIALS AND METHODS

The research was conducted in the Tissue Culture Laboratory at the Indonesian Ornamental Crops Research Institute from January 2007 to March 2008. A randomized complete experiment with 25 replications was designed to accomplish the combination of two factors. The first factor was six commercial cultivars of cut flower chrysanthemum, namely cv. Light Pink Candy, Aicardy, Torpedo, Malaga, President, and Rendezvouz. While the second factor dealt with four formulations of conservation media i.e. modified hyponex medium + 4% sucrose, modified hyponex medium (without sucrose), ½ MS + DMSO 3% + 4% sucrose (as comparison in osmotic pressure method), and ½ MS + 4% (control).

The rooted cuttings of carnation cultivars were collected from commercial nurseries. The cuttings were then, replanted in 15 cm pot and maintained in protected glass house. After 2 weeks, the plants were pinched and the new emerging lateral growths served for explants. The explants were disinfected using chemicals, then inoculated and subcultured into defined medium according to Nugent *et al.* (1991) to obtain uniform plantlets.

After three weeks subculture, 2 node-apical of plantlet was excised into treatment media and placed into growth chamber provided by 18-21^{o}C and 16 h long day. After three days, the plantlets were then preconditioned by lowering the temperature gradually ($\pm$2^{o}C every two days) until constant temperature of 4^{o}C. The viability of plantlets was evaluated and checked every two months during 12 months storage by subculturing the plantlet into induction medium. Prior to subculture, the culture flasks were placed into growth chamber with gradual increase up to 16-18^{o}C (in three days). The observation was

conducted on the survival rate, viability of plantlets after storage and other distinct phenomena related to the treatment being applied.

RESULTS AND DISCUSSION

Analysis of variances of factors studied revealed that there was no significant variation existed on carnation accessions on the survival rate and viability of plantlets on every two months evaluation during 12 months storage. The interaction between conservation media and carnation accessions was not also detected in these parameters.

Survival Rate and Plantlet Viability among the Conservation Media

Percentage of plantlet survival rate and viability of carnation in every two months evaluation during 12 months storage was presented on Table 1. Data on Table 1 showed that plantlet survival rate and plantlet viability were decrease significantly on plantlets inoculated in ½ MS + 4% sucrose compared to those conserved in ½ MS + 3% DMSO + 4% sucrose, modified hyponex medium + 4% sucrose and modified hyponex medium (without sucrose) during the first two months storage. The death of cells corresponding to the death of all plantlets in ½ MS + 4% sucrose media was detected subsequently after four months, while those conserved in the other three media decreased slightly.

The early death of the carnation plantlets conserved in ½ MS + 4% sucrose indicated this medium was not able to protect the plantlets during low temperature storage. The freezing conditions hazardously impacted on the plantlet physiological system. The prolonged period of such extreme condition resulted in loss of cell turgidity and viability, thus finally caused the death of cells and plantlets (Rout and Das, 1997). Though sucrose was also known for preservative additives by inducing partial dehydration of cells (Maddox *et al.*, 1983), these conditions also inferred that the mode of protection could not counteract alone the inherent developmental and physiological differences found in specific plant (Marin and Duran-Villa, 1988).

In contrast with these situation, higher survival rate in longer period of plantlets were found in ½ MS + 3% DMSO + 4% sucrose and modified hyponex + 4% sucrose (Table 1). In these media, carnation plantlets were able to survive up to 10 months storage (Table 1). These conditions inferred that in combination with modified hyponex and supplemented DMSO in MS media, the function sucrose as preservative media acted more effective in increasing of cell retention to low

Table 1. Percentage of survival rate and plantlet viability of carnation accessions among conservation media tested.

Type of conservation media	Percentage of survival rate and plantlet viability of carnation accessions (Observation after ..... months storage*)					
	2	4	6	8	10	12
Survival rates						
½ MS + 4% sucrose (control)	54.2 a	21.4 a	0 a	0 a	0 a	0 a
½ MS + 3% DMSO + 4% sucrose	100 b	92.2 c	72.3 c	53.7 c	17.3 b	0 a
Modified hyponex + 4% sucrose	100 b	89.3 c	75.3 c	43.7 c	12.6 b	0 a
Modified hyponex (without sucrose)	100 b	76.7 b	56.5 b	22.7 b	0 a	0 a
CV (%)	10.41	11.43	13.17	9.47	12.34	8.14
Plantlet viability						
½ MS + 4% sucrose (control)	34.6 a	0 a	0 a	0 a	0 a	0 a
½ MS + 3% DMSO + 4% sucrose	100 b	89.2 c	63.7 c	31.5 c	15.9 b	0 a
Modified hyponex + 4% sucrose	100 b	83.7 c	68.3 c	26.1 c	10.3 b	0 a
Modified hyponex (without sucrose)	100 b	63.1 b	42.7 b	13.4 b	0 a	0 a
CV (%)	12.29	9.31	11.67	10.12	13.53	8.27

* Values followed by different letters in the same column differ significantly at LSD 5%.

temperature condition during storage than single treatment of sucrose. Though the modes of inducing plantlet resistance to low temperature were different, these results also implied that the growth inhibition due to nutrient modification in hyponex medium could counteract the deleterious effect of freezing conditions like membrane protectant, DMSO. Even not properly observed, the plantlet preserved in modified hyponex grew slowly, reflected the physiological inhibitions. In this growing mode, the plantlet usually had smaller cell and thicker cell wall as a result from membrane configuration elicitation (Bonnier and Van Tuyl, 1997). In these situations, the cells were exposed to adjust the electrolyte imbalance between outer and inner part of the cell (Ahn, 1995) and these modified physiological mechanism prevented the injurious level of dehydration due low temperature stress.

Distinction of the Modified Hyponex Conservation Media

Table 1 also showed that carnation plantlets conserved in the media of modified hyponex and modified hyponex + 4% sucrose also showed differences on the plantlet survival rate. Plantlet survival rate conserved in modified hyponex (no sucrose) were only up to 8 months and no plantlets survived here after. In the contrary, plantlets preserved in hyponex + 4% sucrose had longer lasting, 10 months. The failure usage of carbon source by plantlets in ½ MS + 4% sucrose to prevent the optimal growth indicated that the intolerable freezing condition resulted in excessive physiological disturbance and finally the death of plantlet (Figure 1c). Longer plantlet survival rates on hyponex medium than control (½ MS + 4% sucrose) were probably related with the medium composition. Additional microelements which are absent in MS medium and known to have effect on reductase enzyme and membrane stability were supplemented to hyponex medium. This elements may control nitrogen (N) metabolism by binding to a unique metal-protein to form active cofactor (Schwarz *et al*., 2000). These protein bound to cytoskeleton and controlled the activity of nitrate reductase then increase cell membrane stability (Wang *et al*., 2009). These conditions induced higher cell integrity during extreme conditions during low temperature storage at 4^{o}C.

The appearance of roots in plantlets was detected in all carnation cultivars stored in modified hyponex + 4% sucrose and the further root growths were also observed up to 10 months storage (Figure 1b). While those preserved in modified hyponex (no sucrose), the plantlet viability was observed only up

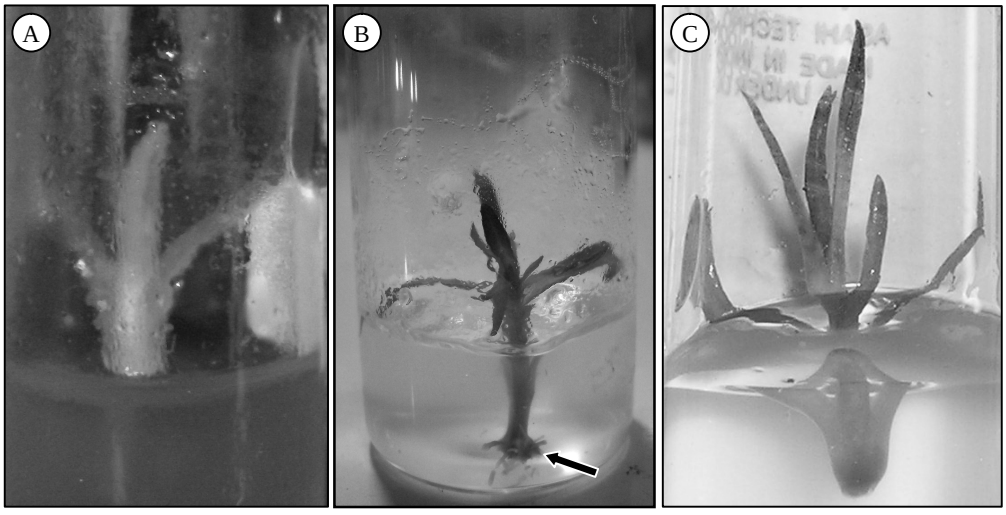

Figure 1. Plantlet performances stored in modified hyponex (no sucrose) (A), the existence of roots were observed on plantlet inoculated in modified hyponex + 4% sucrose (pointing by black arrow) (B), and the plantlet death on the media of ½ MS + 4% sucrose after 4 months at 4^{o}C storage (C).

to 8 months and no root formation in all plantlets was found (Figure 1a). These conditions inferred that root formation was related with prolonged survival of plantlets in which sucrose become the limiting factors in both modified hyponex media. Root formation induced higher and more effective nutrient uptake including carbon from sucrose-containing hyponex medium (Lincy and Sasikumar, 2010). These situations might induce higher resistance of plantlets to low temperature than those stored in the absence of sucrose at modified hyponex medium.

CONCLUSIONS

Medium-term *in vitro* conservation for carnation accessions were successfully conducted up to 10 months using modified hyponex medium + 4% sucrose and ½ MS + 3% DMSO + 4% sucrose without significant variation on all the cultivars tested. In the absence of sucrose, the survival capability of carnation plantlet reached only up to 8 months in low temperature storage at 4^{o}C. In modified hyponex media, the existence of sucrose increased the plantlet resistance to low temperature in prolonged storage. Further efforts to make *in vitro* conservation more efficient, such as the use of nutrient modification and osmotic pressure to overcome the need of low temperature condition were recommended to be investigated.

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