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The Occurrence of Hyperhydricity on Several Carnations (*Dianthus carryophyllus* L.) Cultivars during Low Temperature Storage

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ABSTRACT

The incident of hyperhydricity was a common problem in propagated carnation (*Dianthus carryophyllus* L.) during in vitro culture. Due to its possible relations with the decrease in phenotypic performance of plantlets, the observation on the occurrence of hyperhydricity was conducted on several in vitro conserved carnation cultivars. The research was conducted from July 2007 to August 2008 at The Indonesian Ornamental Crops Research Institute, Cianjur, West Java. A complete factorial experiment with 24 replications was designed to accomplish the combination of two factors. The first factor was six commercial carnation cultivars, namely light pink candy, malaga, opera, white candy, liberty and pink maladi, while the seconds dealt with type of conservation media, i.e. ½MS+DMSO 3%, ½MS+DMSO 3%+3% sucrose and control (½MS+3% sucrose). The results showed that the percentage of hyperhydric plantlet and plantlet viability after in vitro conservation were varied among carnation cultivars. Single treatment of sucrose had the least capacity in inducing plantlet resistance to low temperature conditions during in vitro conservation. Supplemental DMSO postponed the occurrence of hyperhydricity and with the existence of sucrose, higher plantlet viability were achieved.

Key words: carnation (*Dianthus carryophyllus* L.), cultivar, hyperhydricity, plantlet viability, in vitro conservation.

INTRODUCTION

Carnation or "anyelir" (*Dianthus carryophyllus* L.) is an important ornamental cut flower in the world due to their attractive and fragrance inflorescence. The plant belongs to family Carryopillaceae and covers more than 8 genera with 2000 species. The endemic origins ranged from southern Russia to Alpine Greece and the Auvergne mountains of France (Jürgens et al*.,* 2003). Various color and shape of flowers, leaves, flowering response and resistance to pests and disease in the existing cultivars have reflected the complex genetic construction resulted from tight breeding activities from the past. The trend for mono-ideotype cultivar in one hand has improved the preferred characters and incorporated in existing cultivars, while the undesirable traits are swept out (Ben-Yephet et al*.,* 2005). The hastened genetic erosion on such plant has made a great emphasis of preserving genetic resources for future need.

Native from temperate region, the plant had limited life span in the tropics. The maintenance of huge genetic diversity for breeding to important characters in the field were then, very laborious and constrained with some technical problems such as decrease in viability after long clonal propagation and accompanying risks of pest and disease attacks, climatic perturbation and human error (Nugent et al., 1991). These condition were then, made in vitro conservation became an alternative way out in alleviating the limitation in vivo conservation.

The incident of hyperhydricity, morphological, anatomical and physiological disorders of plantlet was a common problem in propagated carnation during in vitro culture. Hyperhydricity was characterized by stressed-glossiness plantlet appearance as results of deficiencies of cell wall edification and associated deficiency in organization of certain tissues such as the vascular bundles and the palisade tissue (Faguel et al*.,* 2008), cuticule and wax deposition and stomata closure, reduced cell-to-cell adhesion resulting in breakability of the organs (Chen *et. al.,* 2006) and lack of chlorophyll accumulation and excess of water in intercellular spaces (Gaspar et al*.,* 1995). Though most of reports referred to genotype dependent, some authors indicated the major causes of hyperhydricity was related with the characteristic of explants and the plantlet environment during in vitro

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culture, such as injury due to the prior dissection of the explants, typical fluxes of Ca and K ions, high relative humidity of the flask atmosphere, ammonium and cytokinine content of the media, and ethylene accumulation (Lai et al*.,* 2005*)*. The abnormal physiological process on hyperhydric plantlet almost all cases decreased phenotypic performance such the loss of organogenic potential which may correspond or be amore advanced form of diminished capacity to organize well structured stem and leaves formations and multiplication rate (Winarto et al*.,* 2004).

The in vitro conservation of carnation was successfully conducted with varying degree of viability among accessions using osmotic pressure method (Halmagyi and Deliu, 2007). Though the effects of DMSO (*dimethylsulfoxide)* as cell protectant on the incident of hyperhydricity has never been reported, the lengthened plantlet survives was presumably related with the action of such permeating agent on cell damage prevention against injurious effects of low temperature condition during in vitro conservation. Related with those facts, the observation on the occurrence of hyperhydricity was conducted on in vitro conserved carnation. In this paper, the affects DMSO conservation media on the abnormal hyperhydric incidence was investigated and further effects on carnation plantlet viability after certain duration of low temperature storage in induction medium was described.

MATERIALS AND METHODS

The research was conducted in the Tissue Culture Laboratory at The Indonesian Ornamental Crops Research Institute, Cianjur, West Java from July 2006 to August 2007. A factorial complete experiment with 24 replications was designed to accomplish the combination of two factors. The first factor was six commercial carnation cultivars, namely light pink candy, malaga, opera, white candy, liberty and pink maladi. The second factor was type of modified conservation media, i.e. ½MS+DMSO 3%, ½MS+DMSO 3%+3% sucrose and control (½MS+3% sucrose).

The rooted cuttings of carnation accessions were collected from commercial nurseries. The cuttings were then, replanted in 15 cm pot and maintained in protected glass house provided by twice a week foliar sprays of 2 g/L complete fertilizer. 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 Budiarto et al*.* (2008) to obtain uniform plantlets.

After three weeks subculture, 2 node-apical of plantlet was excised into treatment media and placed into growth chamber on the temperature of 18-21^{0}C. After three days, the plantlets were then preconditioned by lowering the temperature gradually ($\pm$ 2^{0}C every two days) until constant temperature of 4^{0}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 temperature increase up to 16-18^{0}C (in six days). The observation was conducted on the percentage of hyperhydric plantlet, viability and other distinct phenomena related to the treatment being applied.

RESULTS AND DISCUSSION

Conserved-plantlet of carnation cultivars during low temperature storage

Type of media conservation gave significant influence on hyperhydric plantlet percentage and plantlet viability (Table 1). Similar phenomenon was observed on the carnation cultivars tested. Differences among cultivars were detected on all parameters evaluated during low temperature storage. However, no specific interrelation was found between the type of conservation media and carnation cultivar as far as these concerned.

All the carnation cultivars tested showed hyperhydric symptom during in vitro conservation, though the values were varied among cultivars during 12 months low temperature storage (Table 1). The percentage of hyperhydric plantlets increased in line with the longer low temperature storage. Among the cultivars tested, cv. malaga showed the least hyperhydric plantlets in every 2 months observation up to 12 months in vitro conservation. In accordance with hyperhydric incidences, plantlet viability in induction medium was decreased with the lengthened storage duration. In every 2 months viability observation, cv. malaga still had the higher viable plantlet compared to other cultivars tested.

Table 1. Percentage of hyperhydric plantlet and plantlet viability of carnation cultivars during low temperature storage.

Carnation cultivars	Observation after……..months storage *)					
	2	4	6	8	10	12
	Hyperhydric plantlet (%)					
Light pink candy	1.6 ^{a}	23.6 ^{b}	45.3 ^{b}	74.3 ^{b}	92.5 ^{bc}	100 ^{b}
Malaga	0 ^{a}	8.7 ^{a}	13.6 ^{a}	23.6 ^{a}	32.4 ^{a}	41.2 ^{a}
Opera	2.5 ^{a}	28.3 ^{bc}	42.5 ^{b}	79.3 ^{b}	88.6 ^{b}	93.3 ^{b}
White candy	2.8 ^{a}	27.6 ^{bc}	46.7 ^{b}	83.6 ^{bc}	97.5 ^{bc}	100 ^{b}
Liberty	4.8 ^{a}	33.1 ^{c}	58.7 ^{c}	87.6 ^{c}	100 ^{c}	100 ^{b}
Pink maladi	7.3 ^{a}	36.7 ^{c}	62.8 ^{c}	78.3 ^{b}	93.2 ^{bc}	100 ^{b}
	Plantlet viability (%)					
Light pink candy	100 ^{a}	78.4 ^{ab}	54.3 ^{ab}	40.7 ^{b}	17.7 ^{ab}	4.2 ^{a}
Malaga	100 ^{a}	97.6 ^{c}	88.4 ^{d}	72.3 ^{d}	62.8 ^{d}	54.7 ^{b}
Opera	100 ^{a}	73.2 ^{a}	51.4 ^{a}	31.2 ^{a}	11.2 ^{a}	2.3 ^{a}
White candy	100 ^{a}	74.3 ^{a}	56.7 ^{ab}	37.8 ^{ab}	21.8 ^{b}	6.3 ^{a}
Liberty	100 ^{a}	87.3 ^{b}	62.3 ^{b}	44.2 ^{bc}	27.3 ^{bc}	2.1 ^{a}
Pink maladi	100 ^{a}	91.7 ^{bc}	76.4 ^{c}	56.4 ^{c}	33.4 ^{c}	3.7 ^{a}

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

Different performance among carnation cultivars on the percentage of hyperhydric plantlet and viability might refer to the different genetic constructions in each accession. These differences were also reflected on the ultrastructure microscopy of leaves. Chloroplast structure of normal plantlet of cv. malaga was merely unaffected though the hyperhydric symptoms were initially appeared after 4 months storage, while those on cv. pink maladi, the watery space was systematically increase on the incident of hyperhydricity (Figure 1). These phenomena were also reported on apple (Chakrabarty et al*.,* 2005), coffea (Gatica-Arias et al*.,* 2008*),* sun flower (Faguel et al*.,* 2008) and potato (Turhan, 2004).

The mode of gene action on the hyperhydric-resistant genotype was still in debate up to this moment. Some authors stated that as generally most plant submitted to physical and chemical stresses, the plant possessed antioxidant defense against reactive oxygen species (ROS). These detrimental oxidative agents could attack unsaturated membrane lipids, nucleic acids, enzymes and other cellular structure (Cassi-Lit et al*.,* 1997). In developing system against the ROS, the process involved various antioxidant compounds and a battery of antioxidant enzyme systems including catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), glutathione peroxidase (GPX), glutathione *S*-transferase (GST) and the ascorbate glutathione cycle enzymes (Saher et al., 2004*)*. On the study of normal *Prunus avium* plantlet, the activity of SOD was higher and functioned in disembarrassed the tissue of the accumulated superoxide ions by transforming them into H_2O_2. These H_2O_2 was itself eliminated through catalase involving ascorbate peroxidase, mono-and dehydro-ascorbate reductases and glutathione reductase, and ascorbate and glutathione. On the hyperhydric plantlet, the reduced-activity of such enzymes was almost always linked with DNA composition. It has been suggested then, the decrease in DNA content affected in changes occurring at the cellular level as a result of stress-induced modifications of membrane composition at the abnormal morphology of hyperhydric leaves (Ochatt et al., 2002).

Effects of media conservation on plantlet performance during in vitro conservation

Percentage of hyperhydric plantlet during low temperature storage was varied among plantlets inoculated in media conservation. In general, the occurrence of hyperhydric plantlet was increase in line with the duration of storage. The viability of the survival plantlet in induction medium at every 2 months observation during storage was also in accordance with the incident of hyperhydricity (Table 2).

Table 2. Percentage of hyperhydric plantlet on different conservation media and respective plantlet viability in induction media after low temperature storage.

Conservation media	Observation after……..months storage *)					
	2	4	6	8	10	12
Hyperhydric plantlet (%)						
½MS+DMSO 3%	23.6 ^{a}	31.7 ^{a}	55.4 ^{b}	76.1 ^{b}	97.3 ^{b}	100 ^{a}
½MS+DMSO 3%+3% sucrose	14.5 ^{a}	24.1 ^{a}	41.3 ^{a}	63.7 ^{a}	82.3 ^{a}	97.4 ^{a}
½MS+3% sucrose (control) **)	63.4 ^{b}	89.3 ^{b}	100 ^{c}	-	-	-
Plantlet viability (%)						
½MS+DMSO 3%	97.5 ^{b}	73.8 ^{b}	51.4 ^{b}	36.7 ^{a}	21.4 ^{a}	11.2 ^{a}
½MS+DMSO 3%+3% sucrose	100 ^{b}	93.6 ^{c}	84.5 ^{c}	63.1 ^{b}	47.5 ^{b}	36.8 ^{b}
½MS+3% sucrose (control) **)	75.3 ^{a}	20.3 ^{a}	1.12 ^{a}	-	-	-

Note: *) Values followed by different letters in the same column differ significantly at LSD 5%. **) no plantlet could be observed corresponding the death of plantlet after 8 months storage.

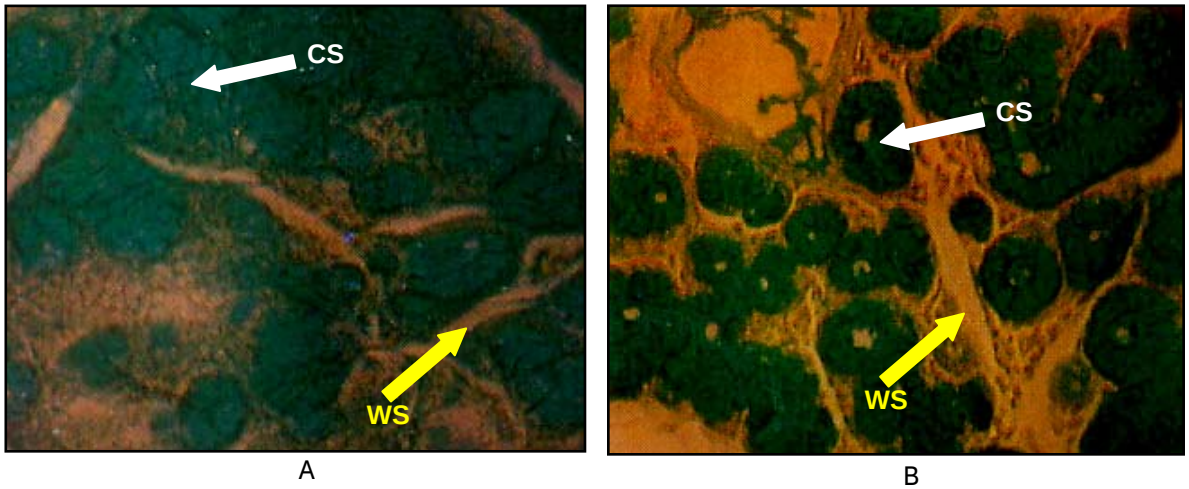

Figure 1. Chloroplast structure on palisade of (a) cv. malaga (normal) and (b) hyperhydric leaves tissue of cv. pink maladi after 4 months low temperature storage. The chloroplast cell and the watery space were pointed by white (CS) and yellow (WS) arrows respectively.

The increase of hyperhydric plantlets in line with storage duration evidenced that the cell biological system was disturbed in prolonged low temperature environment. During these condition, the cell was failed to maintain its turgidity and viability and caused physiological disorder (Manoj et al*.,* 2003). The more extreme impact on the plant corresponds to the early death of plantlet stored in ½ MS+3% sucrose and loss of viability for more than 50% of those stored in ½ MS+3% DMSO after 8 months storage (Table 2). The single treatment of sucrose or DMSO as cell protectant and preservative additives by inducing partial dehydration of cells was a failure in this study as also reported by Kartha et al*.* (1988).

The ambiguous complex mechanism of sucrose and DMSO on increasing plantlet resistance to low temperature was observed when they were existed in combination. The higher plantlet viability in ½MS+DMSO 3%+3% sucrose than those stored in single treatment of DMSO or sucrose on every 2 months examination (Table 3) inferred that the mode of combined action of sucrose+DMSO was depended on plantlet adaptation, though the percentage of hyperhydric plantlet were negligible with the single treatment of DMSO after 12 months. When the cell succeed to adjust osmotic balance between outer and inner by electrolyte accumulation and elicited membrane configuration induced by DMSO, the cell were then able to make use of sucrose to increase resistance to injurious dehydration (Zhao et al*.,* 2005).

CONCLUCIONS

Percentage of hyperhydric plantlet and plantlet viability after in vitro conservation were varied among carnation cultivars. Among the six cultivars tested, cv. malaga had the least hyperhydric plantlets and higher viability post to low temperature storage. Supplemental DMSO were able to postpone the occurrence of hyperhydricity and with the existence of sucrose, higher plantlet viability were achieved. Single treatment of sucrose, however, had the least capacity in inducing plantlet resistance to low temperature storage.

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