ALTERNATIVE IN VITRO MEDIA FOR MEDIUM-TERM CONSERVATION OF CHRYSANTHEMUM (*Dendranthema grandiflora* Tvelve)

(Media Kultur in Vitro Alternatif Untuk Konservasi Jangka Menengah Krisan)

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Abstract

Sufficient genetic diversity and gene pool conservation are needed to serve breeding activities of chrysanthemum. In the tropics, in vitro conservation was expected to be a breakthrough in alleviating the limitation of in vivo methods. A previous study using osmotic pressure methods in low temperature has successfully preserved chrysanthemum via in vitro for medium-term conservation. Modified nutrient compositions were also predicted to have the same impact as far as these concerned. The research was then, conducted to evaluate in vitro conservation of chrysanthemum using media modification in low temperature. The research was carried out on the Indonesian Ornamental Crops Research Institute (IOCRI) from January, 2007 to March, 2008. A randomized completely block design with 25 replications was utilized to accomplish the combination of two factors. The first factor was six commercial chrysanthemum cultivars, namely cv. Puma, cv. Kermit, cv. Town Talk, cv. Snowdown, cv. Yellow Fiji and cv. Saraswati. While the second factor dealt with four formulations of conservation media i.e. ½ MS + DMSO 2,5 % + 4 % sucrose, modified hyponex + 4 % sucrose, modified hyponex (no sucrose) and ½ MS + 4 % sucrose (control). The results showed that the medium term of in vitro conservation for chrysanthemum were successfully conducted up to 12 months using ½ MS + DMSO 2,5 % + 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 planlet survival rates decreased on the media of modified hyponex after 8 months of storage. No significant variation was observed among the chrysanthemum accessions tested.

Keywords : Chrysanthemum (Dendranthema grandiflora), accessions, medium-term conservation, in vitro media, low temperature.

1. Introduction

Chrysanthemum (*Dendranthema grandiflora* Tvelve) is one of popular and major ornamental cut flowers in the world. Native from temperate region and the absence of seed in natural have made this plant has a lot of constraints when planted in the tropical area, like Indonesia. Limited life span in intact conditions, for instance, contributed to the maintenance of active growth plant in base collection at in vivo condition would be very laborious, expensive and accompanying risks associated field such as pathogens, pests, climatic perturbation and human error (Diettrich *et. al*., 1985).

According to some reports, chrysanthemum has been cultivated since long time as a mean of mainly ornamentals. Variation in flower colors and shapes, leaves, flowering responses and pest and disease resistances were known to be the distinct characteristic of this commodity compared to other cut flower ornamentals. These characteristics on the existing commercial cultivars were also reflected the complex combination of parentages and genetic constructions produced from breeding activities from the past up to this moment. While the important characters have been incorporated into the improved cultivars, some lines with unpreferred traits were

swept out. The dynamic trend for mono-ideotype has also hastened the genetic erosion on such plant (da Silva, 2003). Therefore a great deal of emphasis has been placed on the need to preserve genetic resources for the future breeding to important characters as a means of maintaining biodiversity (Poulos, 1993).

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. The growth inhibitor suppressed the cells growth and controlled the plant size in culture flask, while cell protectant kept the cells from all the factors which would affect the viability of the cell/planlet in low temperature during storage (Panis and Lambardi, 2005).

The growth inhibitors fell into several categories i.e osmotic pressure, nutrient modification and growth regulators. Osmotic pressure and nutrient modification were usually applied in this case, since the use of such regulator in almost all cases affected in hormonal imbalance and in the long period, could decrease the viability of the cells (Edson *et.al.*, 1996). Previous study by Budiarto *et al.* (2008) reported that medium-term in vitro conservation of chrysanthemum could be conducted through osmotic pressure method using DMSO (*Dymethil sulfoxide*). With this applications, the planlets could be stored in low temperature up to 12 months without significant decrease of planlet survival rates and viablility after tranferred to induction medium. In some accessions, however, various degree of phytotoxicity was observed in the media containing more than 2.5 % 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). Hyponex medium by Ichihasi (1968) was usually used in this case and with particular modification, this basic medium has been mostly applied in preserving the seed of orchid plants (Nishimura, 1982). Considering the possibility of the use of nutrient modification medium, then in vitro conservation of chrysanthemum was studied. In this paper, medium-term in vitro conservation using modified hyponex medium during low temperature storage was investigated and described.

2. Materials And Methods

The research was conducted in the tissue culture laboratory at the Indonesian Ornamental Crops Research Institute Cianjur,West Javafrom 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. Puma, cv. Kermit, cv. Town Talk, cv. Snowdown, cv. Yellow Fiji and cv. Saraswati. 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 2,5 % + 4 % sucrose (as comparison in osmotic pressure method) and ½ MS + 4 % (control)

The rooted cuttings of chrysanthemum cultivars were collected from commercial nurseries. The cuttings were then, replanted in 15 cm pot and maintained in protected glass house provided by 16 h long day. 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.* (2007) to obtain uniform planlets.

After three weeks subculture, 2 node-apical of planlet was excised into treatment media and placed into growth chamber provided by 18 – 21 ^{0}C and 16 h long day. After three days, the planlets were then preconditioned by lowering the temperature gradually (± 2 ^{0}C every two days) until constant temperature of 4 ^{0}C. The viability of planlets was evaluated and checked every two months during 12 months storage by subculturing the planlet into induction medium. Prior to subculture, the culture flasks were placed into growth chamber with gradual increase up to 16 - 18^0 C (in three days). The observation was conducted on the survival rate, viability of planlets after storage and other distinct phenomena related to the treatment being applied.

3. Results And Discussion

Analysis of variances of factors studied revealed that no significant variation was existed in all chrysanthemum cultivars tested on the survival rate and viability of planlets on every two months evaluation during 12 months storage. The interaction between type of conservation media and chrysanthemum cultivar was not also detected in all parameters observed as far as these concerned.

Survival Rate And Planlet Viability

Percentage of planlet survival rate and viability in every two months evaluation during 12 months storage was presented on Table 1. Data on Table 1 showed that planlet survival rate and planlet viability were decrease significantly on planlets inoculated in ½ MS + 4 % sucrose compared to those conserved in ½ MS + 2.5% 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 planlets in ½ MS + 4 % sucrose media was detected subsequently after four months, while those conserved in the other three media decreased slightly.

Table 1. Percentage of survival rate and planlet viability of chrysanthemum accessions among conservation media tested (*Prosentase planlet hidup dan viabilitas planlet aksesi krisan pada media konservasi yang dicoba*).

Type of conversation media	Observation after Storage months *)					
	2	4	6	8	10	12
Survival rates						
½ MS + 4 % sucrose (control)	64.2 a	11.4 a	0 a	0 a	0 a	0 a
½ MS + 2.5 % DMSO + 4 % sucrose	100 b	92.2 b	91.3 c	89.4 c	83.5 b	79.4 b
Modified hyponex + 4 % sucrose	100 b	94.3 b	93.3 c	90.7 c	85.6 b	80.4 b
Modified hyponex (without sucrose)	100 b	90.7 b	63.5 b	22.3 b	0 a	0 a
CV (%)	8.41	10.43	10.17	7.47	9.34	8.44
Planlet viability						
½ MS + 4 % sucrose (control)	34.6 a	0 a	0 a	0 a	0 a	0 a
½ MS + 2.5 % DMSO + 4 % sucrose	100 b	100 c	92.7 c	84.5 c	79.5 b	72.4 b
Modified hyponex + 4 % sucrose	100 b	100 c	94.1 c	86.1 c	77.3 b	71.3 b
Modified hyponex (without sucrose)	100 b	74.3 b	64.2 b	13.4 b	0 a	0 a
CV (%)	7.29	6.31	8.67	9.12	10.53	7.27

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

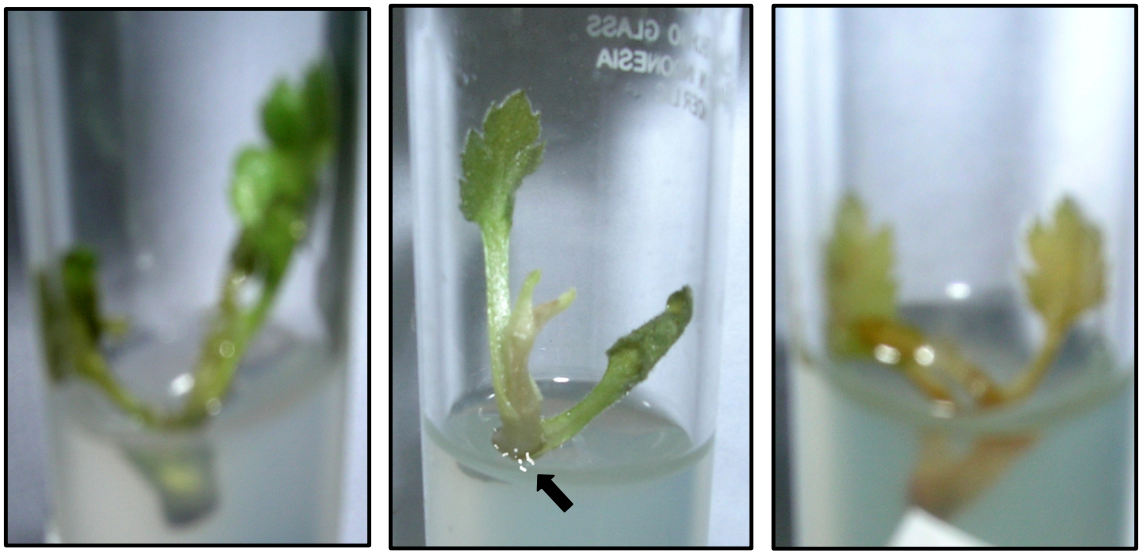
The lower planlet survival rate and viability during the first two months and the death of all planlets after 4 months in ½ MS + 4 % sucrose indicated that during low temperature storage, the cell physiological system was disturbed. The prolonged period of such extreme condition resulted in loss of cell turgidity and viability, thus finally caused the death of cells and planlets (Rout and Das, 1997). These conditions also inferred that though sucrose was also known for preservative additives by inducing partial dehydration of cells and had successfully used for low temperature storage of several plants such as *Acer pseudoplatanus, C. annum, N. tabaccum* and *N. plumbaginifolia* (Whiters and Streets, 1977; Maddox *et al.*, 1982/1983), the mode of protection could not counteract alone the inherent developmental and physiological differences found in specific plant. Several indications were also reported the failure of single treatment of sucrose as protectant during low temperature storage of coffee (Bertrand-Desbrunais *et al.*, 1988), *Picea abis* (Garlene and Dereuddre, 1987) and sweet orange (Marin and Duran-Villa, 1988).

Opposite with these situations, the planlet preserved in ½ MS + 2.5 % DMSO + 4 % sucrose and modified hyponex + 4 % sucrose survived with high viability until 12 months low temperature storage (Table 1). These situations assumed that the

presence of sucrose in combination with modified hyponex and supplemented DMSO in MS media acted more effective in increasing of cell retention to low temperature condition during storage than single treatment of sucrose. Though the modes of inducing plantlet resistance to low temperature were different, these results imply that the growth inhibition due to nutrient modification in hyponex medium could counteract the deleterious effect of freezing conditions like membrane protectant, DMSO. In hyponex medium, the planlet growth was slow as reflected from the smaller cell and thicker cell wall (Bonnier and van Tuyl, 1997) as a result from membrane configuration elicitation (Fukai, 1992). These conditions induced the reduction the cell volume and water content (Fukai, 1990). In these situation, 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 (Hitmi *et. al.,* 2000a).

Distinction Of The Modified Hyponex Conservation Media

Planlet conserved in the media of modified hyponex and modified hyponex + 4 % sucrose also showed differences on the planlet survival rate. Lower planlet survives were found in the absence of sucrose compared to those in the presence of these carbon source on the evaluation after 8 months storage (Table 1). These situations collided with the ambiguous complex mechanism of sucrose in increasing planlet resistance to low temperature during storage. In the media of ½ MS + 4 % sucrose, the planlet could not make use of sucrose in the media to prevent the optimal growth due to excessive physiological disturbance caused by intolerable condition and resulted in the death of planlet (Figure 1c). In contrast, the change of physiological mechanism as affected by nutrient modification in hyponex media could reduce the negative effects of low temperature, the existence of sucrose seemed to be the limiting factor in the prolonged storage-planlet survival.



(a) (b) (c)

Figure 1. Planlet performances stored in (a) modified hyponex (no sucrose), (b) the existence of roots were observed on planlet inoculated in modified hyponex + 4 % sucrose (pointing by black arrow) and (c) the planlet death on the media of ½ MS + 4 % sucrose after 4 months low temperature storage. (*Keragaan planlet terkonservasi pada media (a) hyponex modifikasi tanpa sukrosa, (b) akar yang tampak pada planlet terkonservasi pada media hyponex modifikasi + 4 % sukrosa (yang ditunjukkan dengan panah berwarna hitam) dan (c) kematian planlet pada media ½ MS + 4 % sukrosa setelah 4 bulan penyimpanan pada suhu rendah*)

On the 4 months evaluation, the appearance of roots was detected (Figure 1b) in all chrysanthemum cultivars stored in modified hyponex + 4 % sucrose and the further root growths were also observed up to 12 months storage. While those in the absent of carbon source (modified hyponex), no root formation was found in all cultivars tested (Figure 1a). These conditions inferred that the existence of sucrose in the media was predictably related to the root appearance. Sucrose is well known to be the major carbon source in plant tissue media. Thus, on the existence of sucrose, the protected planlet were sufficiently supplied of organic carbon as the source of energy (Hitmi *et. al.,* 2000b) by maintaining function of absorbing organ. These situations were predictably induced higher resistance of planlets to low temperature than those stored in the absence of sucrose at modified hyponex medium.

4. Conclusions

1) Medium-term in vitro conservation for chrysanthemum accessions were successfully conducted up to 12 months using modified hyponex medium + 4 % sucrose and ½ MS + 2.5 % DMSO + 4% sucrose without significant variation on all the cultivars tested.

2) In the absence of sucrose, chrysanthemum planlet survival rates decreased on the conservation media of hyponex medium after 8 months low temperature storage.

3) In modified hyponex media, the existence of sucrose increased the planlet resistance to low temperature in prolonged storage.

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