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Performance of Four Chrysanthemum [*Dendrathema grandiflora* (Ramat.) Kitam.] Varieties Conserved *In Vitro*

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Four chrysanthemum varieties were conserved *in vitro* at low temperature conditions for 6 mo in four culture media: ½ Murashige and Skoog (MS) + 2.5% dimethylsulfoxide (DMSO) and in Tsuchiya media at ½, ¼ and full strengths. Plantlets of all varieties conserved in MS + 2.5% DMSO had shorter internodes, lesser leaf and internode number and no root formation compared with varieties conserved in Tsuchiya media. Decreasing the nutrient concentration of the Tsuchiya medium to ¼ strength induced more optimum plantlet growth and root development. Mortality in all varieties started at 4 mo storage period. Varieties 'Puspita Nusantara' and 'Tirta Ayuni' conserved in ¼ strength Tsuchiya medium produced the highest number of roots at 6 mo storage. They also had the highest plantlet survival rate. The results showed that successful conservation of chrysanthemum plantlets *in vitro* can be achieved through modification of the nutrients in the culture medium. The findings would greatly help to reduce the maintenance costs of active plant growth in base collections under *in vivo* conditions.

Key Words: Chrysanthemum (*Dendranthema grandiflora*), dimethyl sulfoxide (DMSO), *in vitro* conservation, plantlet performance, percent survival, Tsuchiya medium

Abbreviations: DMSO – dimethyl sulfoxide, IOCRI – Indonesian Ornamental Crops Research Institute, MS – Murashige and Skoog

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflora* [Ramat.] Kitam.) supplies 35% of the world's market demand for cut flowers (Market News Service 2012). Among the Southeast Asian countries, Indonesia, Malaysia and Thailand are competitive producers of chrysanthemums both in cut flower and potted forms. In Indonesia, they have replaced roses as the most marketed cut flowers since 2006. Chrysanthemums vary widely in flower shape, size and color, leaf shape, flowering responses to day length, and resistance to pests and diseases. The fast and dynamic changes in the floriculture market dictate that newly developed cultivars should have unique and advantageous characteristics that are preferred by consumers. Through various breeding methods, more important characters have been incorporated into the improved cultivars while in some lines, the less preferred traits are being wiped out. The dynamic trend in preference for mono-ideotype cultivars has also hastened genetic erosion in plants (Teixeira and Silva 2003). A great deal of emphasis therefore has been placed on the need to preserve genetic resources for the future improvement of important characters to maintain biodiversity (Poulos 1993).

Chrysanthemums are native to temperate regions, and in intact conditions, the plants for cut flower and pot production have a limited life span due to the absence of natural seed formation. In the tropics, the production of high-quality chrysanthemums needs high inputs which include planting them in the highlands under protected environment. This activity has made the maintenance of active plant growth in base collections under *in vivo* conditions very laborious and expensive. In addition, there are risks that are usually associated with field-grown plants such as pathogens, pests, climatic perturbation and human errors (Ozudogru et al. 2010).

D. grandiflora cultivars are complex interspecific hybrids, with an ancestry of 10 or more hexaploid species. Public and private breeding programs put emphasis on continued development and release of asexually propagated cultivars (De Jong 1978). These cultivars show genetic homogeneity following protocols for *in vitro* propagation (Nalini 2012).

In vitro conservation is a promising tool in preserving the base collections of plants especially chrysanthemums. It usually involves the use of cell growth inhibitors and protectants such as dimethylsulfoxide (DMSO) and glycerol. The protectant keeps the cells from factors which would affect the viability of the cells/plantlets at low temperature during storage (Panis and Lambardi 2005). DMSO rapidly permeates into the cells, protecting them better than glycerol. Equimolar concentration of glycerol is less toxic than DMSO, indicating that plants may need different ranges of effective concentrations of DMSO (Klavina et al. 2004).

Modified nutrients as growth inhibitors have the same effect as osmotic pressure. Nutrient modification includes reduction in mineral elements, especially macroelements such as nitrogen (Montalvo-Peniche et al. 2007), sugar (Rakosy-Tican et al. 2012) and vitamins (Engelmann 2010). Reduced nutrient availability and uptake by the plantlets hampered their growth rates during *in vitro* conservation. The method has been successfully applied in the cryopreservation of some plants such as citrus, cassava and potato (Gonzalez-Arnao et al. 2008). A medium used by Tsuchiya (1954) in preserving the seeds of orchid plants has lower concentrations of nitrogen (nitrate, ammonium), potassium, vitamins and carbon source per liter equimolar compared to a common Murashige and Skoog (MS) medium for propagation (Nishimura 1982). Tsuchiya medium has been considered potentially appropriate for *in vitro* conservation of plants with certain modifications. This study aimed to determine the type of medium that is most suitable in conserving *in vitro* four commercial chrysanthemum varieties at low temperature. It also aimed to assess the performance of the varieties at four different periods of storage *in vitro*.

MATERIALS AND METHODS

The four commercial varieties of chrysanthemums used in the study were 'Pasopati', 'Padma Buana', 'Puspita Nusantara' and 'Tirta Ayuni' which were all released by the Indonesian Ornamental Crops Research Institute (IOCRI) in 2007–2009 and are widely grown commercially for the ornamental industry. They were cultured in different media, namely, full strength, ½ strength, and ¼ strength Tsuchiya. The fourth medium, ½ MS + 2.5% DMSO, was included since it has been reported to support conserved plantlets up to 8 mo (Budiarto et al. 2008). All media were supplemented with 15 g L^{-1} sucrose + 15% mannitol. The varieties and media were arranged in a randomized complete block design with three replications.

Initially, uniform rooted cuttings with 2–3 fully expanded leaves and a cutting length of 5–7 cm were obtained from the IOCRI nursery. They were planted in 15-cm pots and maintained in a protected glass house provided with light for 16 h to simulate long-day conditions. Standard cultural practices were performed in maintaining the newly planted cuttings. Two weeks after planting, the shoot tips were pinched and the newly emerging lateral growths served as plant material sources of explants for the *in vitro* culture.

The explants were collected and disinfected following the double sterilization procedure using 1% NaClO for 3 min and rinsed twice with sterile aquadest in every sterilization step. They were then cut into nodal sections and each section was inoculated on solidified ½ MS + 0.5 mg L^{-1} indoleacetic acid (IAA) medium. The cultures were maintained for 3–4 wk at 18 °C and 16 h light culture environment for shoot development. The newly emerging lateral shoot from the explants was then subcultured in ½ MS + 0.1 mg L^{-1} IAA to obtain uniform plantlets. The subsequent plantlet establishment was set at different times, serving as the block of the randomized complete block design which was used in this experiment.

Three weeks after subculture, 2-node apical shoot sections were obtained from the plantlets. Each of the test tubes (1.0 x 5.0 cm) containing 5 mL treatment media was planted with a 2-nodal section reflecting treatment combinations (4 cultivars and 4 media formulations). The test tubes were placed inside a growth chamber at 16–18 ° C with a relative humidity (RH) of 68–70%. The 40-watt TL lamps were arranged in a culture chamber and were approximately 30–40 cm above the culture tubes, 1000 lux for 16 h, long day.

After 3 d, the culture materials were preconditioned by gradually lowering the temperature (2–3 °C every 2 d) until the constant temperature of 4 °C was reached. The cultures were stored under these conditions for 8 mo. Periodically (after 2, 4, and 6 mo of storage), 10 culture materials per replication in every treatment combination were taken out from the growth chamber for data gathering. Samples from all varieties were also set aside to determine their survival rate. The samples were stored up to 8 mo. Plantlet height was measured from the top of the media to the last node at the terminal point of the shoot. Only fully developed leaves were counted. The number and lengths of the youngest and discernible

internodes were recorded. Photographs were taken to visualize the existence and facilitate counting of roots developed by the plantlets. The data were analyzed using ANOVA and the mean comparisons were tested using LSD ($p < 5\%$).

The study was conducted at the Tissue Culture Laboratory of IOCRI, Cipanas, Cianjur, West Java, Indonesia.

RESULTS AND DISCUSSION

Plantlets of the four varieties cultured on four types of conservation media responded differently during each storage period. The final observation on the conserved plantlets was made on the 6th month of the storage period.

Plant Height

All varieties cultured in the different conservation media increased in height with time, although at a slower growth rate in some varieties up to 6 mo of storage. The least increment in height was observed in all varieties cultured in ½ MS + 2.5% DMSO (Fig. 1). In varieties 'Pasopati' and 'Tirta Ayuni', there were no significant differences in heights when plants were stored up to 6 mo in media with DMSO. 'Puspita Nusantara' showed a very slow and insignificant growth rate in the 4th and 6th months of storage (1.12–1.14 cm only). This variety grew more rapidly in ¼ strength Tsuchiya medium. 'Padma Buana' plantlets stored in Tsuchiya medium showed more progressive increases in height, reaching 2.58 cm after 6 mo in ¼ strength Tsuchiya medium. 'Tirta Ayuni' plantlets cultured in ½ strength and ¼ strength Tsuchiya medium grew to about 2.88 cm at the 6th month of the storage period.

The varying growth rates observed in the four varieties reflected the different responses and capacities of each genotype to adapt to a specific medium or circumstance during *in vitro* conservation (Witomska et al. 2008). The growth pressure on the chrysanthemum plantlets was evident in those stored in ½ MS + 2.5% DMSO where there was inhibition on the growth of the shoots (Fig. 2). The inhibitory effect of DMSO was also reported in wasabi (Matsumoto and Nako 1999).

The faster growth rate of the chrysanthemum plantlets conserved in ¼ strength Tsuchiya is related to the higher water content and more dissolved nutrients compared with plantlets conserved in full or ½ strength Tsuchiya (Rossel et al. 1987). The higher nutrient availability in lower nutrient concentration during *in vitro* conservation was also reported in banana by Tokoporo et al. (2013).

Number of Leaves

More leaves were produced by plantlets of all varieties as storage period increased (Fig. 3). The rate of increase in the number of newly developed leaves was slower when the plantlets were conserved in ½ MS + 2.5% DMSO. As in plant height, the DMSO in MS media showed an inhibitory effect in the *in vitro* development of the leaves. This inhibitory effect of DMSO on leaf number and leaf area during *in vitro* storage has also been found in banana (Agrawal et al. 2002).

All varieties showed slower but progressive leaf formation in Tsuchiya conservation media up to the 6th month of the storage period. Leaf development in 'Puspita Nusantara' was slower in ½ strength Tsuchiya and the number of leaves was also lower (3.54) compared with that in the full strength (3.92) and ¼ strength Tsuchiya (4.96). All plantlets cultured in ¼ strength Tsuchiya medium had an average of more than four leaves after 6 mo of storage, indicating that this medium supported the plantlet leaf development during *in vitro* storage. 'Padma Buana' and 'Tirta Ayuni' produced 5.25 and 5.08 leaves in ¼ strength Tsuchiya medium, respectively. Plantlets of 'Pasopati' had the least number of leaves (4.30) at the end of the 6-mo storage period.

Number and Length of Internodes

The increase in number of internodes and leaves in each month of the storage period followed a similar trend. This trend is expected since development of leaves in the shoot apex of the chrysanthemum is on the node. A leaf developed simultaneously with the node on the plant apices, thus the number of leaves in a plantlet was in accordance with the number of nodes (Tariqul Islam et al. 2003). Generally, all varieties had more than four internodes at the 6th month of storage period (Fig. 4). 'Padma Buana' produced the most number of internodes (6.23) followed by 'Puspita Nusantara' (6.21) and 'Tirta Ayuni' (6.14). No interaction between conservation media and varieties for the number of internodes was observed.

In all varieties, elongation of the internodes of the conserved plantlets peaked as early as the 2nd month of storage (Fig. 5). The shortest internode (2.83 mm) was exhibited by the plantlets of 'Tirta Ayuni' stored in ½ MS + 2.5% DMSO, while the longest was observed in 'Pasopati' (5.62 mm) at full strength Tsuchiya . The subsequent internodes of all varieties decreased in length with increased duration of storage in all conservation media. 'Puspita Nusantara' had the shortest internode (2.00 mm) while 'Pasopati' had the longest (3.71 mm) after 6 mo of storage. Within a variety, the length of the internodes did not differ in ½ MS + 2.5% DMSO (except for 'Puspita Nusantara') and in ¼ strength Tsuchiya media at different storage periods. The internode lengths of 'Tirta Ayuni' were comparable in ½ strength Tsuchiya.

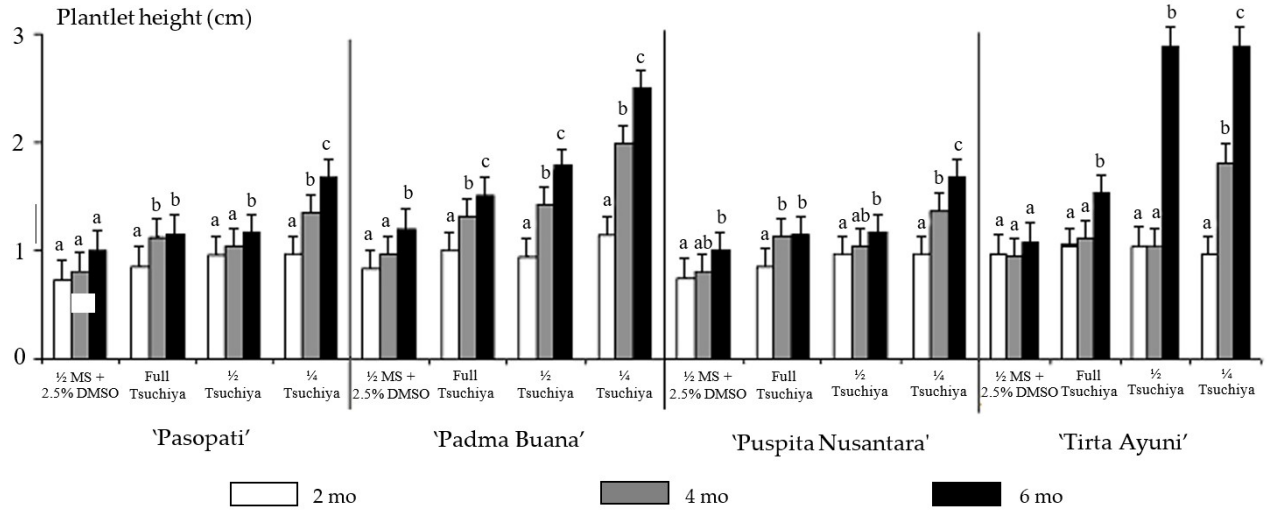

Fig. 1. Heights of plantlets of four chrysanthemum varieties conserved in different media and stored for 2, 4 and 6 mo. Means with the same letters in a conservation medium within a variety/storage period are not significantly different at 5% LSD. Bars represent the standard deviation.

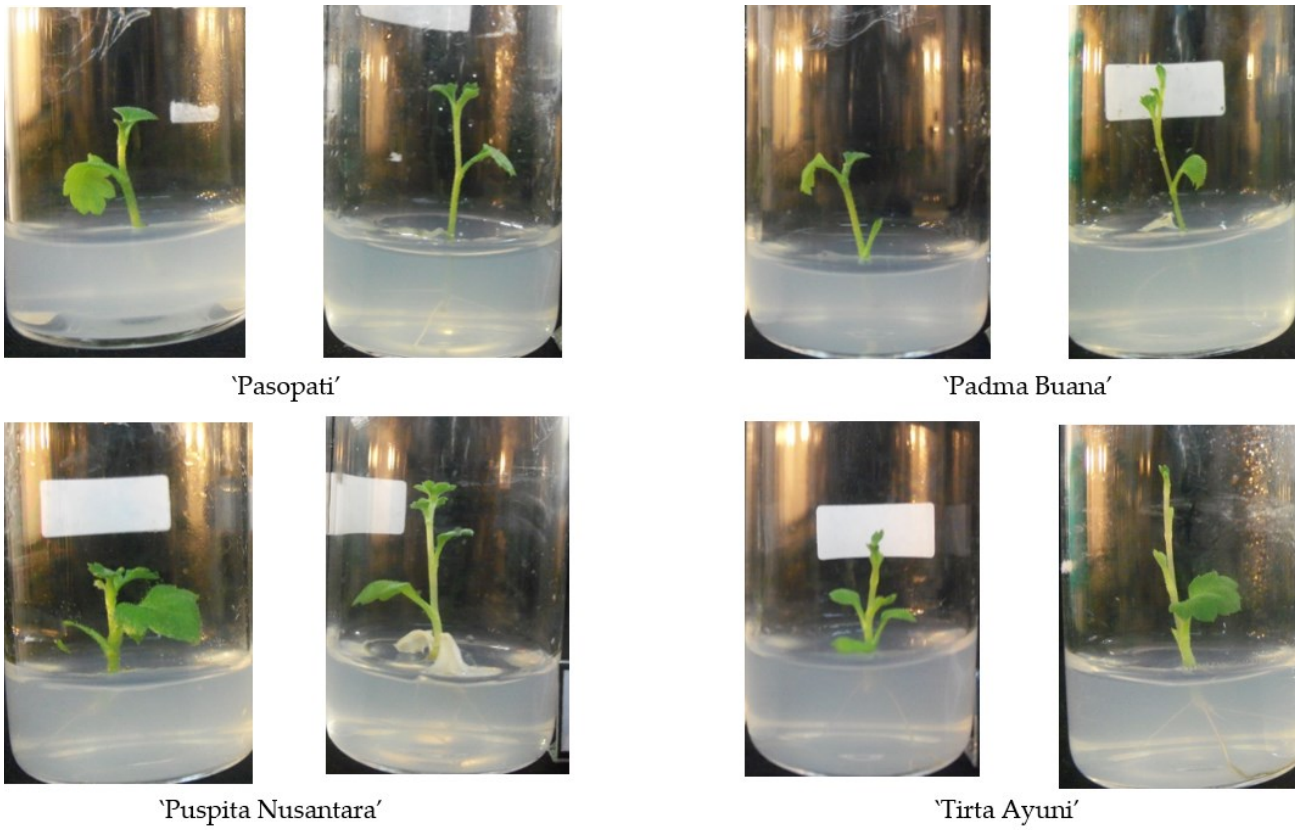

Fig. 2. Shoot development in explants of four varieties of chrysanthemums conserved *in vitro* for 6 mo in ½ Murashige and Skoog + 2.5% dimethylsulfoxide (left of each variety) and ¼ strength Tsuchiya media (right of each variety).

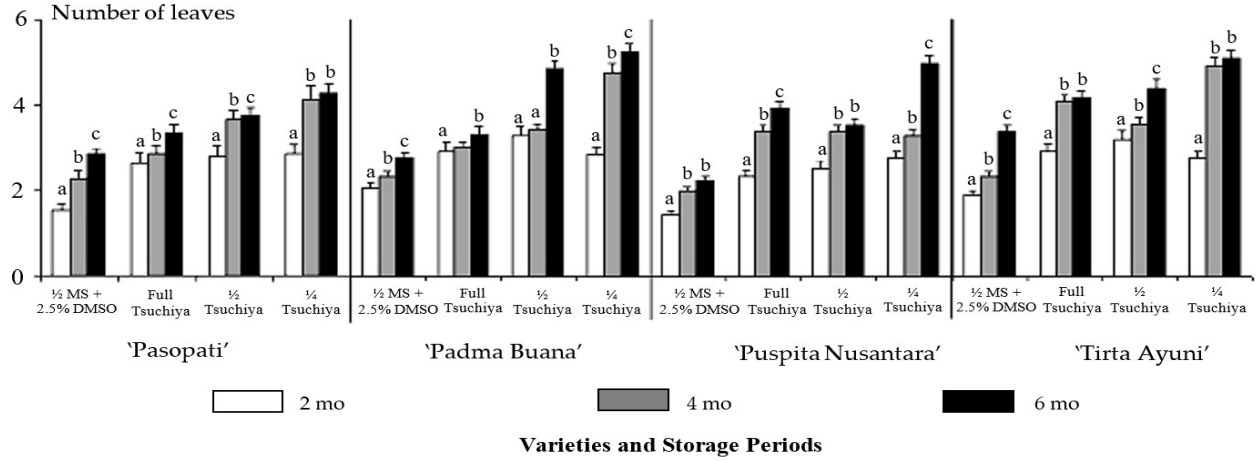

Fig. 3. Number of leaves produced by plantlets of four chrysanthemum varieties conserved in different media and stored for 2, 4 and 6 mo. Means with the same letters in a conservation medium within a variety and storage period are not significantly different at 5% LSD. Bars represent the standard deviation.

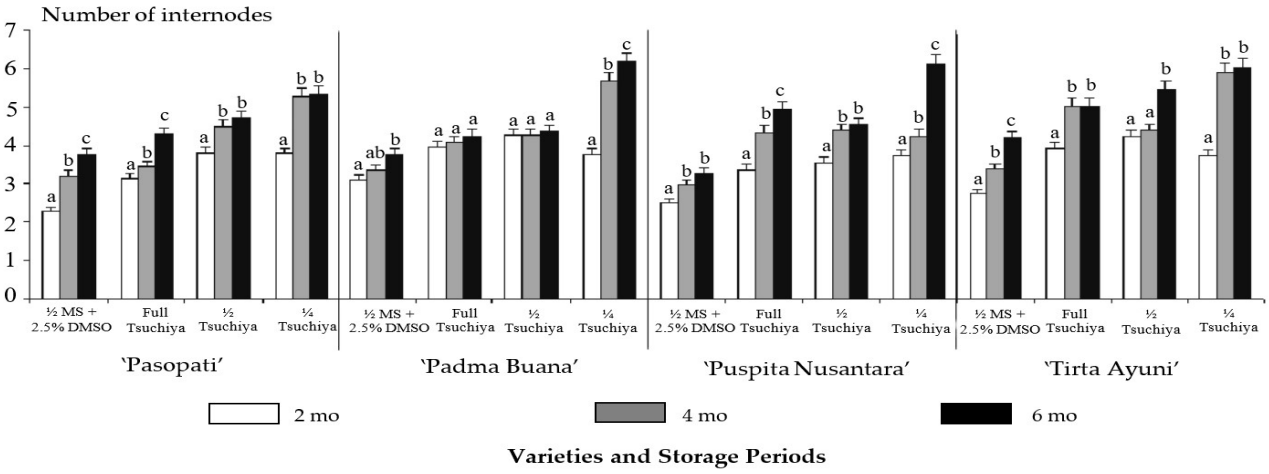

Fig. 4. Number of internodes produced by plantlets of chrysanthemum varieties conserved in different media and stored for 2, 4 and 6 mo. Means with the same letters in a conservation medium within a variety/storage period are not significantly different at 5% LSD. Bars represent the standard deviation.

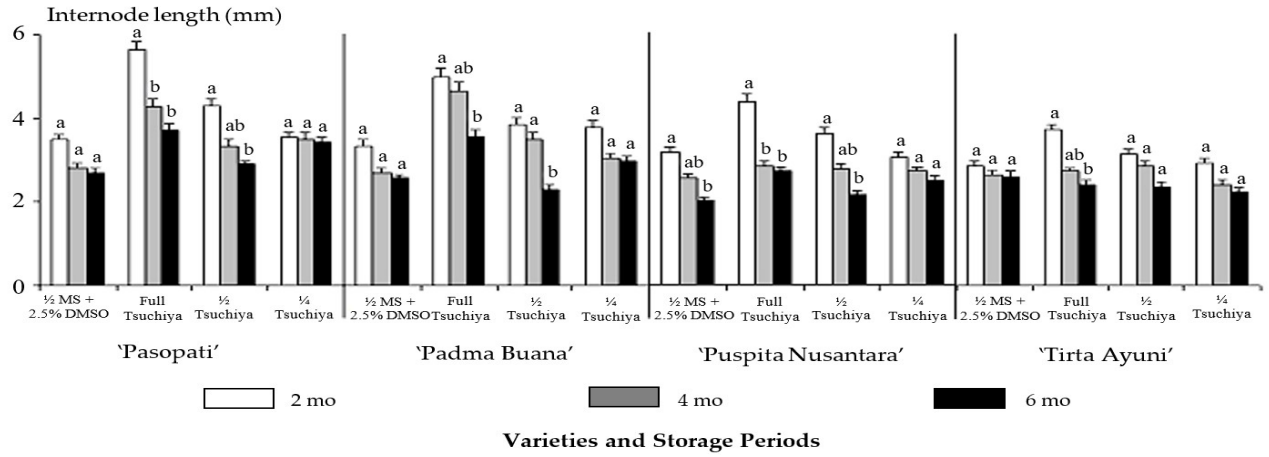

Fig. 5. Internode lengths of plantlets of chrysanthemum varieties conserved in different media and stored for 2, 4 and 6 mo. Means with the same letters in a conservation medium within a variety/storage period are not significantly different at 5% LSD. Bars represent the standard deviation.

Growth retardation of plantlet during *in vitro* conservation due to DMSO was also reported in cassava (Escobar et al. 1997). Growth inhibition was related to the change in cell membrane permeability to prevent chilling injury during exposure to low temperature. DMSO induced membrane thinning and increased membrane hydrophobic features (Gurtavenko and Anwar 2007) and limited the flow of water and ions into the cell (Binder 1981). The limited flow of nutrient ions into the cell then retarded plantlet growth. In *Spilanthes acmella*, there was a decrease in cell volume and cell enlargement in the plantlets during *in vitro* conservation (Joshi and Jadhav 2013). Several reports also indicated that DMSO as a mutagen carrier may enhance undesirable somaclonal variation after *in vitro* storage (Wang et al. 2008).

Significant differences were found in the interaction between varieties and conservation media in all varieties after 6 mo of storage (Table 1). The genetic constitution of 'Tirta Ayuni' made it less sensitive to growth pressure in terms of internode length under different conservation media. It had more tolerance and wider adaptation under growth pressure conditions during *in vitro* storage. A similar phenomenon was observed in shoot development of macadamia nut (Gitonga et al. 2010) and sweet potato (Arrigoni-Blank et al. 2014) genotypes during *in vitro* propagation in different conservation media.

Root Formation

During the first 2 mo of storage, there was no obvious root development in any plantlet in all treatment combinations. In all Tsuchiya media, the conserved chrysanthemum varieties started forming roots during the 4th month of storage and more roots were discernible at the 6th month (Fig. 6). Fewer roots, however, were produced in full strength Tsuchiya. Plantlets of all varieties conserved in ¼ strength Tsuchiya media produced the most number of roots after 6 mo. 'Tirta Ayuni' produced 3.76; 'Puspita Nusantara', 3.46; 'Padma Buana', 3.42; 'Pasopati', 3.31.

All conservation media were not supplemented with any plant growth regulator. In all varieties tested, there was no root formation in the plantlets conserved under ½ MS + 2.5% DMSO up to the 6th month of storage. The mode of action of DMSO in root formation is still unclear although the inhibition of root development is presumably affected by the lack of auxin which was needed for cell development. DMSO is known to block the polar transport of auxin by trafficking and dislocating the efflux protein carrier (Shibasaki et al. 2009); therefore, it retards the disposition of auxin on the targeted growth site (Reed et al. 1998). This condition implies that the hormonal regulation on the plantlet depended on the endogenous hormone present within the plant body expressed specifically in root formation in plantlets in conservation media. Auxin is synthesized in the leaves (Zhao 2010) and transported basipetally to the root (An et al. 2001). Plantlets conserved in ¼ strength Tsuchiya had more leaves and in the absence of an auxin transport inhibitor, it was sufficiently deposited on the targeted basal stem to induce root formation. The highest number of roots was produced in this conservation medium in all varieties (Fig. 7).

A summary of the vegetative parameters of the varieties in all treatment combinations is shown in Table 2. The heights of all varieties were the least and the values were not significantly different when conserved in ½ MS + 2.5% DMSO. Within each variety, the values in this medium did not differ from those obtained by plantlets conserved in full strength Tsuchiya. The same results were observed in the number of leaves produced, except for 'Puspita Nusantara', and in the length of internodes, except for 'Tirta Ayuni'.

The number of roots produced in ½ and ¼ strength Tsuchiya media did not show any variation among the varieties. For full strength Tsuchiya, the number of roots produced by 'Pasopati' and 'Padma Buana' did not differ; the same results were observed in 'Puspita Nusantara' and 'Tirta Ayuni'. In all varieties, no roots were developed in media with DMSO, leading to the death of the plantlets.

Table 1. Interaction effects of chrysanthemum variety and conservation media on internode length of the conserved plantlet after 6 mo of storage.

Chrysanthemum Variety	Conservation Medium[1]			
	½ MS + 2.5% DMSO	Full Strength Tsuchiya	½ Strength Tsuchiya	¼ Strength Tsuchiya
'Pasopati'	2.70a Y	3.71b Y	2.88a Y	3.42b Z
'Padma Buana'	2.55ab Y	3.58c Y	2.29a X	2.96b YZ
'Puspita Nusantara'	2.00a X	2.71c X	2.17ab X	2.50abc XY
'Tirta Ayuni'	2.62a Y	2.42a X	2.37a XY	2.25a X

[1]All media were supplemented with 15 g L^{-1} sucrose + 15% mannitol. Values in the same row followed by different letters in lower case differ significantly at 5% LSD. Values in the same column with different letters in upper case differ significantly at 5% LSD.
MS – Murashige and Skoog, DMSO – dimethyl sulfoxide

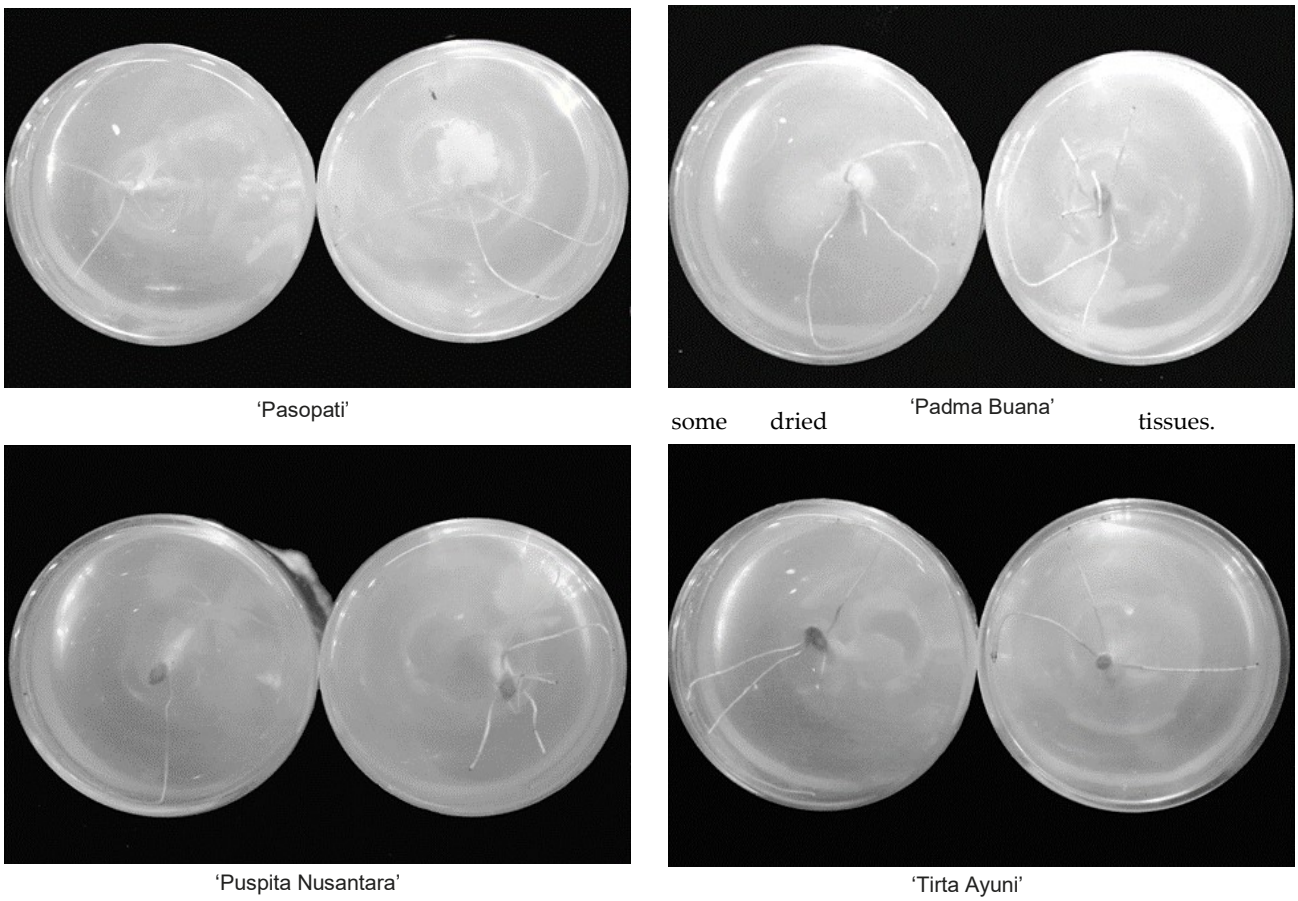

Fig 6. Root formation in plantlets of four chrysanthemum varieties conserved in ¼ strength Tsuchiya media for 6 mo.

A higher nutrient concentration in full strength Tsuchiya induced slower growth rate as observed in plantlet height, number of leaves and roots. With the presence of mannitol, higher nutrient concentration might induce higher osmotic pressure on the media and could have prevented optimum nutrient absorption by the conserved plantlets. On the other hand, in media with lower ion, the osmotic retention was lower and the conserved plantlet might have made use of the nutrient to support higher growth rate. Similar findings on plantlet height, number of nodes and root number and length were observed in line with the decreasing concentration of salt nutrient and osmotic agent in *Smallanthus sonchifolius* under *in vitro* conservation (Skalova et al. 2012).

Plantlet Survival

Mortality in plantlets conserved in ½ MS + 2.5% DMSO started at 4 mo in all varieties (Fig. 8). 'Tirta Ayuni' and 'Puspita Nusantara' had the lowest survival rate (37.50%) while 'Padma Buana' had the least dead plantlets with 58.33% survival. The plantlets eventually died. The dead plants were necrotic, yellow to brown, wilted and with some dried tissues.

The survival rate of the plantlets in some varieties decreased in 6 mo. The decrease was most noticeable in 'Padma Buana' where only 8.33% of the plantlets survived in full strength Tsuchiya. In the same medium, the survival rate of 'Pasopati' dropped from 80.34% to 20.83%. Comparing all varieties, the survival rate of plantlets conserved in ½ strength and ¼ strength Tsuchiya was higher. There was 100% survival rate in plantlets of 'Tirta Ayuni' conserved in ½ and ¼ strength Tsuchiya for 8 mo. 'Puspita Nusantara' had 87.5% and 100% survival in ½ and ¼ strength Tsuchiya, respectively.

The percentage survival of the plantlets of chrysanthemum during *in vitro* conservation was shown to be related to the existence of roots. Plantlets with higher number of roots tended to have higher percentage of survival after storage. Plantlets conserved in ½ MS + 2.5% DMSO did not have roots and the nutrient uptake was dependent on the capacity of the cells at the stem base (Charoensub and Phansiri 2004). The limited mineral uptake by the stem base did not maintain the minimum active growth conservation and resulted in early death.

Plantlets with more roots absorb the nutrients in the

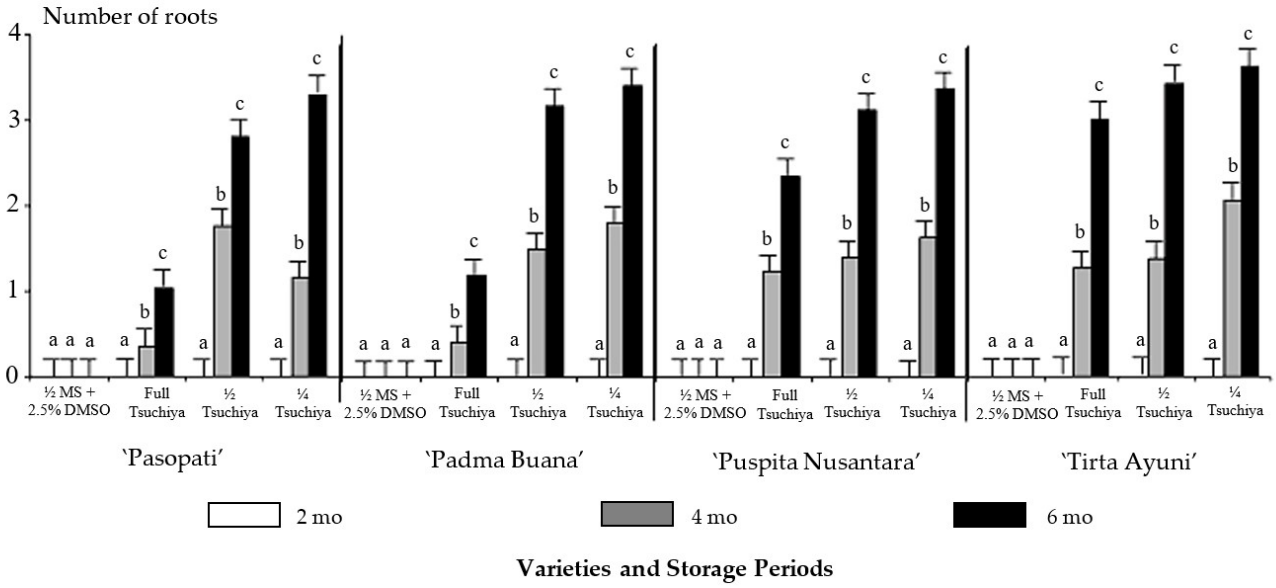

Fig. 7. Number of roots of four chrysanthemum varieties conserved in different media and stored for 2, 4 and 6 mo. Means with the same letters within a variety/storage period are not significantly different at 5% LSD. Bars represent the standard deviation.

Table 2. Vegetative characteristics of four chrysanthemum varieties conserved *in vitro* in four different media for 6 mo.[1]

Variety	Conservation Medium[2]	Plantlet Height (cm)	No. of Leaves	Internode		No. of Roots
				No.	Length (mm)	
'Pasopati'	½ MS + 2.5% DMSO	1.18ab	2.83abc	3.79ab	2.70bcde	0.00a
	Full strength Tsuchiya	1.29abcd	3.38abcd	4.31bcd	3.71g	1.02ab
	½ strength Tsuchiya	1.65bcde	3.75bcdef	4.71cde	2.88de	2.82cd
	¼ strength Tsuchiya	2.10ef	4.30defg	5.34efg	3.42fg	3.31cd
'Padma Buana'	½ MS + 2.5% DMSO	1.22abc	2.75ab	3.82ab	2.55bcde	0.00a
	Full strength Tsuchiya	1.55abcde	3.33abcd	4.30c	3.58g	1.18b
	½ strength Tsuchiya	1.85cde	4.83efg	4.41bcd	2.29abc	3.17cd
	¼ strength Tsuchiya	2.58f	5.25g	6.23h	2.96ef	3.42cd
'Puspita Nusantara'	½ MS + 2.5% DMSO	1.00a	2.25a	3.33a	2.00a	0.00a
	Full strength Tsuchiya	1.14ab	3.92bcdefg	5.02cdef	2.71cde	2.41c
	½ strength Tsuchiya	1.17ab	3.54abcde	4.61bcde	2.17ab	3.21cd
	¼ strength Tsuchiya	1.88de	4.96fg	6.21h	2.50abcde	3.46cd
'Tirta Ayuni'	½ MS + 2.5% DMSO	1.08ab	3.38abcd	4.28bc	2.62bcde	0.00a
	Full strength Tsuchiya	1.54abcde	4.15cdefg	5.13def	2.42abcd	3.12c
	½ strength Tsuchiya	1.88de	4.42defg	5.56fgh	2.37abcd	3.54d
	¼ strength Tsuchiya	1.88de	5.08fg	6.14gh	2.25abc	3.76d

[1]Values within a column followed by different letters differ significantly at 5% LSD.
[2]All media were supplemented with 15 g L^{-1} sucrose + 15% mannitol.
MS – Murashige and Skoog, DMSO – dimethylsulfoxide

media to support growth during prolonged period of storage at low temperature, thus resulting in increased plantlet survival (Shatnawi et al. 2011). The positive relationship between higher survival rates and presence or existence of roots in *in vitro* conserved chrysanthemum plantlets was also reported on several plants such as carnation, coffee and stevia (Holobiuc et al. 2004–2005; Kartha et al. 1981; Shatnawi et al. 2011).

CONCLUSION

Chrysanthemum varieties conserved *in vitro* in four different media showed varied degrees of growth responses. Plantlets of all varieties conserved in MS + 2.5% DMSO medium had the shortest internodes, suppressed leaf development and no roots, which resulted in high mortality rate. The dead plants were necrotic, yellow to brown, wilted and with some dried tissues.

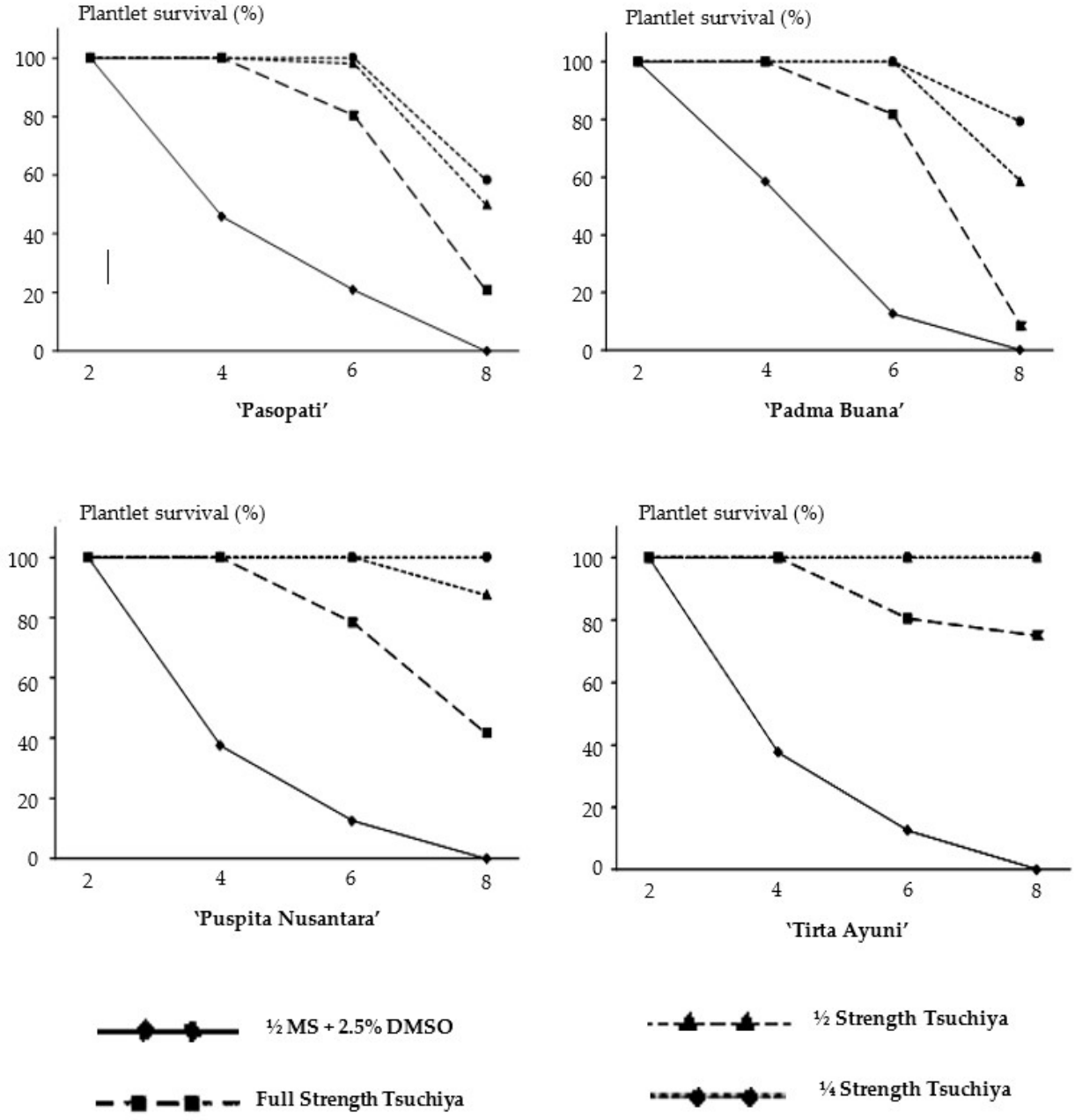

Fig. 8. Percentage survival of plantlets of four chrysanthemum varieties conserved in four different media and stored at four different periods. MS – Murashige and Skoog, DMSO – dimethyl sulfoxide

Elongation of the internodes of the conserved plantlets in all varieties reached its peak at the 2nd month of storage. The subsequent internodes decreased in length with increased duration of storage in all conservation media. Varieties conserved in different strengths of Tsuchiya media showed better vegetative performance compared with those cultured in MS + 2.5% DMSO medium. There was no significant interaction between varieties and conservation media in all varieties at the 6th month of the storage period.

The number of roots produced in ½ strength and ¼ strength Tsuchiya media did not show any variation among the varieties. 'Tirta Ayuni' and 'Puspita Nusantara' had the highest survival percentage even up to the 8th month of storage when conserved in ¼ strength Tsuchiya medium. They also had the highest number of roots which greatly contributed to their higher percentage of survival.

In this study, chrysanthemum plantlets were successfully conserved *in vitro* for 6 mo through nutrient modification of the culture media and maintenance under low-temperature conditions. The results showed that Tsuchiya medium with various strengths can be used for preserving the genetic diversity in chrysanthemum under *in vitro* conditions. The method may reduce the limitations of *in vivo* germplasm collection especially when planting is done under tropical conditions. A continuation of this study was done to determine the fidelity of the conserved plantlets under field conditions and the results are also published in this journal.

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