PERBAIKAN MEDIA INDUKSI YANG TERSELEKSI TERHADAP INDUKSI PEMBENTUKAN KALUS PADA KULTUR ANTHER ANTHURIUM DAN STUDI HISTOLOGINYA PADA PEMBENTUKAN KALUS. Studi ini telah dilakukan pada laboratorium kultur jaringan, Balai Penelitian Tanaman Hias Pacet – Cianjur sejak bulan Februari hingga Oktober 2008. Tujuan penelitian ini adalah untuk mengoptimalkan media terseleksi dalam induksi pembentukan kalus, mengungkap asal-usul sel yang membentuk kalus hasil kultur anther dan proses pembentukan tunasnya. Media terseleksi yang diperbaiki adalah 1) MMS-TBN yang mengandung 0,5 mg/l TDZ, 1,0 mg/l BAP dan 0,01 mg/l NAA (Medium Winarto, WM) dan 2) MMS III yang ditambah dengan 1,5 mg/l TDZ, 0,75 mg/l BAP dan 0,02 mg/l NAA (Medium Winarto dan Rachmawati, WRM). Perbaikan media menginduksi potensi pembentukan tunas dan penambahan 2,4-D pada konsentrasi 0,5 mg/l dan penurunan kekuatan medium dari kekuatan penuh, setengah, seperempat, seperdelapan, seperenambelas dan nol. Percobaan faktorial disusun menggunakan rancangan acak kelompok.

ABSTRAK

Application of anther culture and/or microspore culture in ornamental crops till now is still limited. The technique was reported in several plants such as on lily (van den Bulk et al., 1992; Han et al., 1997), tulip (Tanaka and Ito, 1981 and 1982; van den Bulk et al., 1994), sunflower (Saji and Sujatha, 1998), petunia (Mohan-Jain and Bhalla-Sharin, 1996), Camellia japonica (Pedroso and Pais, 1996). While in Araceae, especially in anthurium, its application was very limited. In Araceae, double haploid plant production was tried in Spathiphyllum via ovule culture (Eeckhaut et al., 2001), but number of double haploid plant produced was very low.

INTRODUCTION

Application of anther culture and/or microspore culture in ornamental crops till now is still limited. The technique was reported in several plants such as on lily (van den Bulk et al., 1992; Han et al., 1997), tulip (Tanaka and Ito, 1981 and 1982; van den Bulk et al., 1994), sunflower (Saji and Sujatha, 1998), petunia (Mohan-Jain and Bhalla-Sharin, 1996), Camellia japonica (Pedroso and Pais, 1996). While in Araceae, especially in anthurium, its application was very limited. In Araceae, double haploid plant production was tried in Spathiphyllum via ovule culture (Eeckhaut et al., 2001), but number of double haploid plant produced was very low.

Winarto and co-workers tried and initiated experiments in anther culture of anthurium from 2003. Two important points determined from the previous results were 1) two different calluses were regenerated from anther culture and 2) Modified Murashige and Miller Syngonium (MMS) containing 1.5 mg/l TDZ, 0.75 mg/l BAP and 0.02 mg/l NAA (Winarto and Rachmawati medium, WRM) was the most potential medium to induce callus and its regeneration (Rachmawati et al., 2005; Winarto and Rachmawati, 2007). In a comparative study it was also found that MMS supplemented with 0.5 mg/l TDZ, 1.0 mg/l BAP and 0.01 mg/l NAA (Winarto medium, WM) was another potential medium in anther culture of anthurium (Winarto et al., 2009a).

Two different calluses derived from anther that a part of them often showed different colors in their performance (green and yellow in Tropical and Amigo cultivars; red-purple and yellow in Carnaval cultivar) and growth types (slow and fast) interested to be studied in detail via their histology. From the histological study it was expected that callus origin could be clearly known. In the first time it was hypothesized that the slow growth callus was initiated from microspore cells and the faster one was derived from anther wall and/or connective tissue cells. Important role of the study in in vitro cultures was generally carried out to support and strengthen research results. The study was applied in anthers of Vitis rupestris (Altamura et al., 1992), in androgenesis of rice (Oryza sativa L.) (Nakano and Maeda, 1989; Mandal and Gupta, 1996), in callogenesis and organogenesis of Curcuma zedoaria.

Kata-kata kunci: Perbaikan, media, kalus, anther, histology dan anthurium.
Roscoe (Mello et al., 2001), in petiole derived callus of *Amorphophallus rivieri* Durieu (Hu et al., 2005).

Improvement of induction culture medium in callus initiation and formation via application of 2,4-D and/or increasing its concentration was actually contributed in increasing morphogenic response of the anther walls and connective tissues to divide actively and produce callus as stated by Rodrigues et al., (2004) in soybean anther culture. Enhancing callus initiation due to 2,4-D treatment was also recorded by Thengane et al. (1994) in anther culture of sunflower, Arzate-Fernandez et al. (1997) in lily, Oggeema et al. (2007) in sweet potato, Kumar and Kanwar (2007) in *Gerbera jamesonii*. Strengthening medium capacity in callus induction was also recorded successfully by reduction of the medium strength to be half, quarter, or others as reported by Hoque and Arima (2002) in callus induction of water chestnut (*Trapa Japonica* Flerov), Chen et al. (2005) in *Bupleurum kaoi* Liu, Jabeen et al. (2006) in *Aconitum heterophyllum*, Wang and Bao (2007) in *Viola wittrockiana*. Furthermore application of 2,4-D and reduction of medium strength in anthurium anther selected medium expected could improve medium capacity in stimulating high response of the anthers cultured for producing callus.

The objectives of the present investigation were 1) to improve selected induction culture medium in anther culture of anthurium by 2,4-D application and reduction its strength on callus formation; and 2) to reveal from which cell and/or tissue actually the regenerated calluses on anther culture derived.

**MATERIALS AND METHODS**

**Preparation of aseptic plant materials**

This research was conducted at Tissue Culture Laboratory of Indonesia Ornamental Crops research Institute from February to October 2008. *Anthurium andreanum* Linden ex André c.v. Tropical used in the experiments was grown in plastic bags (30 cm in diameter) in a mixture of rice-hush + bamboo moss + cicas (1:1:1, v/v/v) as a potted medium. The plants were placed in the glass house and maintained optimally via fertilization (application of 1 gr NPK (15:15:15) per plant monthly and using 1.5 ml/l of Growmore once two weeks) and watering them. Spadixes with 50% of its pistil in receptive condition were harvested from the plants.

In the first step, spadixes were placed under tap water for 30-60 minutes to reduce high contamination, followed by immersing in pesticide solution (1% of benomil and bactomycin) for 30 minutes and then rinsed by distilled water for 5-6 times with 5 minutes each. After first step sterilization, the spadixes were brought into laminar air flow cabinet for next sterilization. Explants were soaked in 2% of sodium hypochlorite (NaOCl) plus 5 drops of Tween 20 for 5 minutes, 1% of NaOCl added by 5 drops of Tween 20 for 10 minutes, followed by rinsing in distilled sterile water for 5-6 times with 5 minutes each. The sterile spadixes were used in all experiments.

**Improvement of anthurium anther selected media via addition of 2,4-D**

Sterile spadix was put in sterile petridish, cut the transition area and used for anther isolation. Petals were then removed carefully and anthers were isolated using tissue culture blade. Top part of anther (halve anther) without filament was isolated and cultured in the culture medium tested. All isolation activities (anther isolation steps) are under stereo microscope.

Selected media improved in the study were 1) MMS-TBN containing 0,5 mg/l TDZ, 1,0 mg/l BAP and 0,01 mg/l NAA (Winarto medium, WM) and 2) MMS III supplemented with 1,5 mg/l TDZ, 0,75 mg/l BAP and 0,02 mg/l NAA (Winarto and Rachmwawati medium, WRM). Improvement of medium in callus induction was carried out by addition of 2,4-D in 0.5 mg/l. Media tested in the experiment were 1) MMS-TBN without 2,4-D (WM), 2) MMS III without 2,4-D (WRM), 3) MMS-TBN added by 0,5 mg/l 2,4-D (WM-D) and 4) MMS III supplemented with 0,5 mg/l 2,4-D (WRM-D). All media contained 30 g/l sucrose and were adjusted at pH 5.8 before thier sterilization in 121°C, 15 kPa for 20 minutes.

The experiment was arranged using a randomized completely design with four replications. Each treatment consisted of 3 bottles and each bottle contained 6 anthers. All cultures were incubated in the dark condition for ± 2 months; afterward the cultures were put under fluorescent lamp (13 μmol.m⁻².s⁻¹) for 12 h photoperiod until callus formed. Parameters observed in all experiments were 1) potential growth of anther (PGA, %), 2) percentage of anther regeneration (PAR, %), and 3) number of callus formed per replication (NCF). The first parameter was recorded one month after culture initiation; second one was noted 2.0 months after culture initiation; and third one was collected 3.0 months after culture initiation. Quantitative data were analyzed by analysis of variance (ANOVA) using SAS program Release for Windows 6.12. In cases where significant differences were obtained (p=0.05), Duncan’s Multiple Range Test (DMRT) was used for comparison between means.
Improvement of anthurium anther selected media via medium strength reduction

Anther isolation steps were carried out as previously described in experiment 1 mentioned above. Selected media improved in the study were 1) WM and 2) WRM. The media were built thier capacity in callus formation through reduction of medium strength of full, half, quarter, one eighth, one sixteenth, and zero strength. The factorial experiment was arranged using a randomized complete block design with four replications. Each treatment consisted of 3 bottles and each bottle contained 6 anthers. Culture condition, observation and quantitative data analysis were conducted as previously described in experiment 1. All media contained 30 g/l sucrose and adjusted to pH 5.8 prior to sterilization in 121°C, 15 kPa for 20 minutes.

Histological study of callus formation

The histological study was carried out to recognize the original cells and/or tissues that produced callus derived from anthers. Callus explants were sampled at 0, 1, 2, and shoot initiation stage. The specimens were then fixed in the FAA solution (formalin: glacial acetic acid: 50% ethanol, 5:5:90 (v/v/v)) for 48 h and dehydrated in ethanol series (30, 50, 75, 95, 100% (v/v)) twice for 30 min in each step. After dehydration the explants were immersed in xylene: paraffin in different ratios (75:25, 50:50, 25:75, 0:100) for an hour per ratio and in the final level explants were soaked in 100% paraffin for one night. In the next step samples were embedded with paraffin. Serial sections (10-15 µm) were made with a rotary microtome model 820 Spencer and peaces of specimen sections were mounted with 10% of albumin-glycerin on objective glass. The specimens were double stained with 1% acid-fuchsin and 0.05% toluidine blue or safarin and fast green. Finally, the specimens were mounted with DPX mountant. The slides were then observed under the microscope and photographed.

RESULTS AND DISCUSSION

Improvement of anthurium anther selected media via addition of 2,4-D

Callus formation in anther anthurium was initiated 1.0-1.5 months after culture initiation. The initiated callus continued to grow in different shapes and sizes. Number of callus formed varied from 1-6 calluses per replication. A part of callus derived from anthers turned to brown and died. The anther browning of anthurium was as reported and discussed in detail by Winarto et al. (2009b).

Improvement of two selected media with 2,4-D, in fact, gave different effects on callus induction. In WRM, supplementation of 2,4-D in the medium reduced potential growth of anther down to 59% with 34% anther regeneration and 1.8 calluses formed per replication. While in WM, addition of 2,4-D (0.5 mg/l) increased average value of all parameters observed (Table 1). Based on the highest average of data recorded it was clearly known that WRM was the most suitable induction culture medium in callus formation of anthurium anthers. The medium exhibited the highest results and significantly different compared to other media.

Existence of 2,4-D in anther culture of anthurium clearly gave two different effects in two selected media tested i.e. strengthening and weakening effect of them. The effect was probably caused by different concentrations of all medium components as reported by Winarto et al. (2009). Increasing NH₄NO₃ concentration from 500 mg/l to 750 mg/l and KNO₃ from 1250 mg/l to 1750 mg/l, enhancing myo-inositol from 110 mg/l to 125 mg/l and thiamine-HCl from 0.5 mg/l to 0.55 mg/l in WM to WRM improved medium capacity in inducing regenerative response of anther explant and role of the components in stimulating regenerative capacity was also stated by George (1993). Therefore addition of 2,4-D in the medium caused reduction in callus formation potential (weakening effect). With 1.5 mg/l TDZ, 0.75 mg/l NAA and 0.02 mg/l NAA without 2,4-D, WRM was to be the most suitable medium for anther culture of the plant. In another study reported that the existence of 2,4-D in the medium reduced cell viability in Doritaenopsis (Mishiba et al., 2001), caused microspore plasmolysis and did not improve androgenesis (Rodrigues et al., 2004).

Improvement of anthurium anther selected media via medium strength reduction

The study also revealed that though the experiment result was not as high as the previous study, but the WRM was still the most appropriate induction culture medium on callus induction compared to WM-D (Table 2). Improvement of them via reduction of their strength gave a significant effect on percentage of anther regeneration and number of callus formed, however there was no interaction response on both treatments. The result of the experiment also strengthened and gave evident that WRM kept being the most optimal medium on callus induction in anther culture of anthurium. Interesting
results were recorded on reduction medium strength in callus formation. The reduction influenced number of callus induced gradually and reached the highest effect on one eighth strength with 54% of anther growth, 29% anther regeneration and 1.8 number of callus produced per replication and then reduced till the zero strength. From the experiment it was revealed that the one eighth strength was the most suitable reduction of selected medium strength in obtaining high result and exhibiting significant different compared to others. The result gave evident that medium strength reduction stimulated high effect on callus formation in anther culture of anthurium as recorded by Hoque and Arima (2002) in Water Chestnut (*Trapa japonica* Flerov), Chen et al. (2007) in vitro *Bupleurum kaoi*, Wang and Bao (2007) in pansy (*Viola wittrockiana*).

Hoque and Arima (2002) reduced MS medium to half-strength supplemented with 2.7 mM 2,4-D, 108.0 mM casein hydrolyzate, and 10.8 mM phloroglucinol to support maximum callus induction. A seventy-five percent primary callus induction rate was obtained from the explants cultured on half-strength MS medium containing 4 mg/l 2,4-D for 8 weeks in darkness (Chen et al., 2007). High callus induction of pansy was recorded on a half-strength MS medium supplemented with 0.45 μmol l⁻¹ 2,4-d plus 8.9 μmol l⁻¹ BA (Wang and Bao, 2007). While totipotent calli of a Paphiopedilum hybrid (*Paphiopedilum callusum* ‘Oakhi’ × *Paph. lawrenceanum* ‘Tradition’) were easily induced from seed-derived protocorms on a 1/2 strength Murashige–Skoog medium plus 1–10 mg l⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.1–1 mg l⁻¹ 1-phenyl-3-(1.2.3-thiadiazol-5-yl)urea (TDZ) (Lin et al., 2000).

### The histological study of callus formation

Surprisingly result was observed in histological studies on callus formation derived from half anther cultured in the selected media. The histological study results revealed that anther wall cells were actually the origin cells that grew and formed anther primordium.

### Table 1. Effect of selected-media improved by addition of 2,4-D on callus induction in anther culture of anthurium

| Selected Media | 2,4-D (mg.l⁻¹) | Potential growth of anther (%) | Percentage of anther regeneration (%) | Number of callus formed |
|---------------|---------------|--------------------------------|---------------------------------------|-------------------------|
| MMS-TBN       | 0.0           | 45.0 c                         | 32.5 b                                | 1.8 a                   |
| MMS-III       | 0.0           | 80.8 a                         | 48.6 a                                | 2.7 a                   |
| MMS-TBN       | 0.5           | 63.8 b                         | 42.7 ab                               | 2.4 a                   |
| MMS-III       | 0.5           | 58.5 b                         | 33.6 b                                | 1.8 a                   |
| Coefficient variation | 13.57 | 11.67                         | 11.18                               |

Means followed by the same letter in the same column are not significant different based on Duncan Multiple Range Test (DMRT, *p*=0.05).

### Table 2. Effect of selected induction culture media on callus formation

| Selected Media | 2,4-D (mg.l⁻¹) | Potential growth of anther (%) | Percentage of anther regeneration (%) | Number of callus formed |
|---------------|---------------|--------------------------------|---------------------------------------|-------------------------|
| WRM           | 0.0           | 48.3 a                         | 18.8 a                                | 1.2 a                   |
| WM-D          | 0.5           | 46.2 a                         | 11.1 b                                | 0.7 b                   |
| Coefficient variation | 10.89 | 20.67                         | 19.43                               |

Means followed by the same letter in the same column are not significant different based on Duncan Multiple Range Test (DMRT, *p*=0.05).

### Table 3. Reduction effect of selected medium strength on callus initiation in anther culture of anthurium.

| Medium strength | Potential growth of anther (%) | Percentage of anther regeneration (%) | Number of callus formed |
|-----------------|--------------------------------|---------------------------------------|-------------------------|
| Full strength   | 37.5 c                         | 4.2 b                                 | 0.3 b                   |
| Half strength   | 42.7 bc                        | 7.3 b                                 | 0.4 a                   |
| Quarter strength| 52.1 ab                        | 11.5 b                                | 0.7 b                   |
| One eighth strength | 58.4 a                 | 29.2 a                                | 1.8 a                   |
| One sixteenth strength | 54.2 ab               | 25.0 a                                | 1.5 a                   |
| Zero strength   | 38.5 c                         | 12.5 b                                | 0.8 b                   |
| Coefficient variation | 10.89 | 20.67                         | 19.43                               |

Means followed by the same letter in the same column are not significant different based on Duncan Multiple Range Test (DMRT, *p*=0.05).
produced callus. Fifteen to twenty days after culture initiation the cells changed to be responsive and competent to medium components and exogenous plant growth regulators utilized in the selected media as also reported by Nicuta et al. (2005) in anther culture of *Brassicaoleracea*. The anther wall cells were then to become morphogenic, overcame dedifferentiation and divided actively. Shape cell altered from oval and longer in size to be short and round and called as meristematic cells (Figure 1c-d). The cells divided actively in all direction and produced callus (Figure 1e-g). The callus derived from anther wall cells continued to grow and enlarge in shape, size and volume and clearly observed 2.0-3.0 months after culture initiation (Figure 1f-g).

It could also be reported that high morphogenic anther wall cells were determined from anther wall cells located between connective cells and internal epidermis anther sacs (indicated by red circles in figure 1a-c). From four anther sacs containing microspore cells that were divided into two main parts (contain 2 sacs each) it was only a main part that indicated high morphogenic response, produced callus and grew faster than another main part (Figure 1c-e). Growth direction was indicated by white arrows (Figure 1d-f).

From the study it was also well recognized that during callus formation microspore cells did not grow and do nothing. The cells kept and maintained in their position and existence from the early culture initiation till 2-3 months later (Figure 1a-f, green arrows). The condition was actually caused by morphogenic response of anther wall cells. The morphogenic response of anther wall and a few of connective tissue cells became the greatest obstacle in androgenesis of anthurium microspores as reported by Rodrigues et al. (2004) in soybean anther culture and a bit different situation was observed in connective tissue cells. The cells also showed drastic slow growth and were generally in stagnant position. A part of them was distributed in different direction due to active growth of anther wall cells (Figure 2e-g). In anther culture of rice, callus masses was originally regenerated from connective tissue (Maeda et al., 1978), while in anther culture of *Vitis rupestris*, caulogenesis was regenerated from all anther tissue except endothecium (Altamura et al., 1992).

Calluses derived from half-anthers cultured on the selected media continued its growth for several months after culture initiation. After one to three times of explant sub-culture initial apical meristem developing was observed (Figure 2h-i). In the stage meristimoid area consisting of densely cytoplasmic cells was formed. In the next step the initial apical meristem developing grew continually to be initial shoots 4.5-6.0 months after culture initiation indicated by further growth of the meristimoid area for further cell division till arising small protrusions of tissue which gradually became green and organized into a growing point (Figure 2j). Finally the shoot buds development was clearly observed (Figure 2k-l).

**CONCLUSION**

Entirely it can be concluded that the highest callus induction was clearly established in WRM. The medium stimulated potential growth of anther (PGA) up to 81% with 49% of percentage of anther regeneration (PAR) and 2.7 number of callus formed per replication (NCF). Significant improvement in callus formation was also recorded by reduction of medium strength of WRM to one eighth compared to others. The reduction induced PGA up to 58% with 29% of PAR and 1.8 NCF. From histological studies it was well recognized that regenerated callus on half anthers cultured on selected medium was originated from anther wall cells that were morphogenic in response to plant growth regulators and medium components. The morphogenic response of anther wall cells caused primarily on no androgenesis effect in microspore cells.

**ACKNOWLEDGMENTS**

I would like to express my grateful thank to the Indonesia Toray Science Foundation for the opportunity given to me in pursuing a 2008 Research Grant to carry out research in title: *Several improvement treatments in anther culture of anthurium*. I would like also to express my high appreciation to Fitri Rachmawati, Dewi Pramanik, Euis Rohayati, Supenti for their cooperation and helps during research activities conducted at tissue culture laboratory of Indonesian Ornamental Crops Research Institute.

**REFERENCES**

Ahmad, Z. 1993. A Study of The Chemical Manipulations Involved in The Clonal Propagation of Pistacia Vera. Thesis. Institute of Chemistry University of the Punjab, Lahore. 235 pages

Altamura, M. M., A. Cersosimo, C. Majoli and M. Crespan. 1992. Histological study of
Figure 1. Histological studies of callus formation derived from anther till shoot initiation. **a**-longitudinal section of anther in initial culture, **b**-cross section of anther in initial culture, **c-d** – initial morphogenic response of anther 20 – 35 days after culture initiation, **f-g** – regenerated and developed callus derived from anther 2.0-3.0 months after culture initiation, **h-i**-initial apical meristem developing 4.0-4.5 months after culture initiation, **j**-initial shoot developing 4.5-6.0 months after culture initiation. **k-l** – developed shoots more than 6 months after culture initiation. Red arrows = connective tissue/cells, blue arrows = high morphogenic anther wall cells, green arrows = microspore cells, **amd** – apical meristem developing, **am** – apical meristem, **vcd** – vascular cells developing, **vc** – vascular cells, **ylp** – young leaf primordia, **yl** - young leaf. Blue bars = 0.11 mm, green bars = 0.28 mm, red bars = 0.001 mm
embryogenesis and organogenesis from anthers of *Vitis rupestris* du Lot cultured in vitro. *Protoplasma* 171:134-141

Chen, U.C., F.S. Chueh, C.N. Hsia, M.S. Yeh and H.S. Tsay. 2005. Influence of 2,4-dichlorophenoxyacetic Acid on Leaf Callus Induction, proliferation and Saikosaponin Formation of in vitro *Bapleuraum kaoi* Liu, Chao et Chuang. *Crop, Environment & Bioinformatics* 2: 39-49

Eeckhaut, T., S. Werbrouck, J. Dendauw, E. van Bockstael and P. Debergh. 2001. Induction of homozygous *Spathiphyllum wallisii* genotypes through gynogenesis. *Plant Cell, Tissue and Organ Culture*. 67: 181-189.

Han, D.S., Y. Niimi and M. Nakano. 1997. Regeneration of haploid plants from another cultures of the Asiatic Iribid lily ‘Connecticut King’. *Plant Cell, Tissue and Organ Culture*. 47: 153-158.

Herman. 2000. Control of Browning during Explant Establishment. *Agricell Report. Agricell@AOL.COM*. Access date: 6 November 2008

Hoque, A. and S. Arima. 2002. Overcoming Phenolic Accumulation during Callus Induction and In Vitro Organogenesis in Water Chestnut (*Trapa japonica* Flerov). *In Vitro Cell. Dev. Biol.—Plant* 38:342–346.

Hu, J. B., J. Liu, H. B. Yan and C. H. Xie. 2005. Histological observations of morphogenesis in petiole derived callus of *Amorphophallus rivieri* Durieu in vitro *Plant Cell Rep* 24: 642–648EL

Jabeen, N., A.S. Shawl, G.H. Dar, A. Jan and P. Sultan. 2006. Callus induction and organogenesis from explants of Avonitum heterophyllum-medicinal plant. *Biotechnology* 5(3): 287-291.

Kumar, S and J.K. Kanwar. 2007. Plant Regeneration from Cell Suspensions in *Gerbera jamesonii* Bolus. *Journal of Fruit and Ornamental Plant Research* 15: 157-166

Lin, Y.H., C. Chang and W.C. Chang 2000. Plant regeneration from callus culture of a *Paphiopedilum* hybrid. *Plant Cell, Tissue and Organ Culture* 62(1): 21-25

Mandal, N. and S. Gupta. 1996. Studies on histomorphological course of events during androgenesis of rice (*Oryza sativa* L.) *Phytomorphology* 46(2): 99-107

Maeda, E., V.M. Villalobos and T. Sugiiura. 1978. Fine structure of the regenerating cells in *Fragaria* anther cultures. *Japan. J. Breed*. 28(2): 143-146.

Mello, M.O., M. Melo and B. Appezzato-da-Glória. 2001. Histological analysis of the callogenesis and organogenesis from root segments of *Curcuma zedoaria* Roscoe. *Brazilian Archives of Biology and Technology*. 44(2): 197 – 203.

Mishiba, K., T. Okamoto and M. Mii. 2001. Increasing ploidy level in cell suspension cultures of *Doritaenopsis* by exogenous application of 2,4-dichlorophenoxyacetic acid. *Physiologia Plantarum* 112(1): 142 - 148

Mohan Jain, S. and N. Bhalia-Sarin. 1996. Haploidy in Petunia. In: *In Vitro* Haploid Production in Higher Plants. Mohan Jain, S., S.K. Sopory and R.E. Veileux (Eds.) Volume 5: p. 53-71. Kluwer Academic Publishers. Dordrecht/Boston/London.

Nakano, H. E. Maeda.1989. Cyto histological studies on callus formation and its regeneration in anther culture of *Oryza sativa* L. *Japanese J. Breed*. 38:3: 288–300.

Nichita, N.D., I.N. Toma and G.I. Ghiorgi. 2005. Morphogenetical and histological studies of “In Vitro” anther cultures of *Brassica oleracea* L. Genetica and Biologe Moleculara. TOM V: 111-118.

Ogema, J.N., M.G. Kinyua and J.P. Ouma. 2007. Optimum 2,4-D concentration suitable for embryogenic callus induction in local Kenyan sweet potato cultivars. *Sian Journal of Plant Sciences* 6(3): 484-489.

Pedroso, M.C. and M.S. Pais. 1996. Anther and microspore culture in *Camelina japonica*. In: In *Vitro* Haploid Production in Higher Plants. Mohan Jain, S., S.K. Sopory and R.E. Veileux (Eds.) Volume 5. p: 89-108. Kluwer Academic Publishers. Dordrecht/Boston/London.

Rachmawati, F., B. Winarto and A. Purwito. 2005. Kultur Anther pada Anthurium (*Anthurium andreanum* Lindem ex André). Thesis. Departemen Agronomi dan Hortikultura. Fakultas Pertanian. Institut Pertanian Bogor. 146 Halaman.

Raghavan, V. 2004. Role of 2,4-dichlorophenoxyacetic acid (2,4-d) in somatic embryogenesis on cultured zygotic embryos of *arabidopsis*: cell expansion, cell cycling, and morphogenesis during continuous exposure of embryos to 2,4-d. *American Journal of Botany* 91(11): 1743–1756.

L.R. Rodrigues, L.R., B. de C. Forte, J.M.S. Oliveira, J.E.A. Mariath and M.H. Bodanese-Zanettini. 2004. Effects of light conditions and 2,4-D concentration in soybean anther culture. *Plant Growth Regulation* 44: 125–131

Saji, K.V. and M. Sujatha. 1998. Embryogenesis and plant regeneration in anther culture of sunflower (*Helianthus anuus* L.) *Euphytica* 103: 1-7.

Svetleva, D.L., and P. Crino. 2005. Effect of ethyl methanesulfonate (ems) and n-nitrose-n-ethyl urea (enu) on callus growth of common bean.
Winarto et al.: Improvement of culture media on callus induction and formation

Journal of Central European Agriculture. 6(1): 59-64.

Tanaka, I. and M. Ito. 1981. Studies on microspore development in Lilaceous Plant III. Pollen tube development in Lily pollens cultured from the uninucleate microspore stage. Plant and Cell Physiology. 22(1):149-153.

__________. 1982. Additional mitosis induced by bromodeoxyuridine in explanted tulip microspores. Plant Science Letters. 27: 37-42.

Thengane, S.R., M.S. Joshi, S.S. Khuspe, and A. E Mascarenhas. 1994. Anther culture in Helianthus annuus L., influence of genotype and culture conditions on embryo induction and plant regeneration. Plant Cell Reports 13:222-226

Van den Bulk. R.W., H.P.J. de Vries-van Hulten, J.B.M. Custers and J.J.M. Dons. 1994. Induction of embryogenesis in isolated microspores of tulip. Plant Science. 104: 101-111.

Wang, J. and M.Z. Bao. 2007. Plant regeneration of pansy (Viola wittrockiana) ‘Caidie’ via petiole-derived callus. Scientia Horticulturae. 111(3): 266-270

Winarto, B dan F. Rachmawati. 2007. Teknik kultur anther pada pemuliaan anthurium. J. Hort. 17(2): 127-137.

Winarto, B., F. Rachmawati, N.A. Mattjik, A. Purwito, dan B. Marwoto, 2009a. Pengembangan formulasi medium dasar untuk kultur anther anthurium. Dikirim untuk dipublikasikan pada Jurnal Stigma Fakultas Pertanian, Universitas Andalas, Padang, pada tanggal 20 April 2009.

B. Winarto, F. Rachmawati dan D. Pramanik. 2009b. Pengaruh cara isolasi, jenis dan konsentrasi agar terhadap induksi pembentukan kalus pada kultur anther anthurium. Dikirim untuk dipublikasikan pada Jurnal Hortikultura, Pusat Penelitian dan Pengembangan Hortikultura Jakarta, pada tanggal 23 Januari 2009.