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[Home](#) [Journals](#) [Journal of Engineering and Applied Sciences](#) [Archive](#) [Volume 12 Issue 15, 2017](#)

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[CURRENT ISSUE](#) | [ARCHIVE](#) | [EDITORS](#) | [GUIDE TO AUTHORS](#) | [SUBMIT A MANUSCRIPT](#)

ARCHIVE >> Volume 12 Issue 15, 2017

[Differentiation of Agarwood Oil Quality Using Support Vector Machine \(SVM\)](#)

Humuerah Jantan, Ihsan M. Yassin, Azlee Zabidi, Nurhaila Ismail and Megat Syahirul Amin Megat Ali

[Flow Visualization Study of Wing-Tip Vortex for a Wing with Cavity](#)

Tey Shen Xi, Noorfazreena Mohammad Kamaruddin and Zulfaa Mohamed-Kassim

[Positioning of Fused Deposition Features on Primitives](#)

Patharawul Suphama, Kuntinee Maneeratana and Ratchalin Chanchareon

[Optimum Number of DC Fan as a Cooling Medium for Photo Voltaic \(PV\) System](#)

A.R. Amelia, Y.M. Irwan, M. Irwanto, W.Z. Leow, M.H. Mat and I. Safwati

[Landscape Design for Housing Areas: A Review](#)

Mohd Ramzi Mohd. Hussain, Fitriyadia Mohd. Shahli, Izawati Tukiman and Ismawi Hj. Zen

[Numerical Analysis of Torsionally Loaded Drilled Shafts Near an Embankment Slope in Cohesionless Soils](#)

Alina Irsainova, Aigul Mussabayeva, Sudheesh Thiyya Kkand and Jong Kim

[Characterizations of Activated Carbon Produced from Bagasse and Banana Stem using H3PO4 as Activating Agent](#)

Erni Misran, Seri Maulina, Sari Farah Dina, Surya Adi Anggi Harahap and Ahlun Nazar

[The Factor Affecting Malaysian Citizens Satisfaction with Open Government Data](#)

Mohammed Shihab Ahmed, Massudi Bin Mahmuddin and Nor Idayu Binti Mahat

[Performance Management in Crude Palm Oil Industry using Analytical Hierarchy Process](#)

Abdul Talib Bon, Silvia Firda Utami Utami and Sukono

[Software Project Management Approaches for Monitoring Work-In-Progress: A Review](#)

Hamzah Ali Alawi Al-Aidaros and Mazni Omar

[Supply Chain Management in Service Companies \(Case Study in Indonesia\)](#)

Chairul Saleh, Syeh Assery, Sabihaini and Sri Suryaningsum

[Interdependence Psychology of Indonesian Village](#)

Cholichul Hadi and Ismail Suardi Wekke

[Differentiation Strategy of Islamic Micro Finance Institutions in Malang](#)

Fuad Ibrahim, Christea Frisdiantara and Ismail Suardi Wekke

[A Little Aspect of Misbehavior in Organization \(Case Study in Indonesia\)](#)

Nur Feriyanto, Syeh Assery, Chairul Saleh and Sri Suryaningsum

[Assesment of Non-Linear Interpolators to Construct Digital Elevation Models](#)

Julian Garzon Barrero, Gonzalo Jimenez Cleves and Francisco Luis Hernandez Torres

[On Representation of Discrete Information of Temporal Databases in the Continuous Form](#)

Gennady V. Averin, Anna V. Zviagintseva, Maria V. Shevtsova and Liliana N. Kurtova



Design and Principles of Iranian Calligraphy

Faeze Tahery, Fahimeh Daneshgar and Mansoor Hesami Kermeni

Efficiency of Colleges at Prince Sattam Bin Abdulaziz University, Al Khafj (A Comparative Study using Data Envelopment Analysis)
Teg Alam

The Explants of Platelet Induction using Auxin and Cytokinin Shortly after the Gamma Ray Irradiation and the Gripped Poly Ethylene Glycol

Ari Wijayani and Rina Sri Iestari

Problem Solving Application on Guidance and Counseling Teachers for Bullying Victim Students

Faizah Binti Awad and Ismail Suardi Wekke

Analysis of Financial Performance before and after Implemented ISAK No. 29 in Mining Companies

Sutoyo and Sujatmika

Oblique Compression Shock Wave and Shock Wave Polars

Vladimir N. Uskov and Pavel V. Bulat

Measurement of Thermic and Electric Properties of the System $x\text{AgI}-(1-x)\text{NH}_4\text{I}$ at High Temperatures

J.A. Trujillo, A. Garcia-Muriel and R.D. Ortiz

Tetroleit Transform Based Satellite Image Enhancement

R. Durga Singh

Application of Wireless Sensor Networks in Real Time Patient Health Status Monitoring System

P. Ravichandran

A Potential Finite Element Scheme for Elastic Solids

G. Vijayaganth

An Overview of 3-Dimensional Angiography for Computational Fluid Dynamics

N. Jayakumar

Design and Thermal Analysis of a Rocker Arm

A.R. Sivaram

Zero Production Maintainable Craft for Coastal Marine Shielded Regions

K. Saravana Kumar

Coastal Region Offshore Transportation and System

Samson Joseph

Telemetry based Quality Control and Directional Wave Information Improvement in Real Time

P. Ravichandran

Construction on Study of Marine Network Information Security Protection System

R.N. Raju

Nautical Chart Understanding for Autonomous Surface Ship Operations

R. Durga Singh

Hybrid Dynamic Fuzzy Cognitive Maps for Self-Directed Mobile Direction-Finding

P. Ravichandran

Communication Block Design in Marine Simulator: A Review

R.N. Raju

A Review of Finite Element in Bone Microstructures

G. Vijayaganth

Finite Element Method a Tool in Machine Design

J. Harish Kumar

Mechanization Hydro Turbine Runner Design

M. Vinoth Kumar

Submerged Sensor and Experimental Authentication of Separate Seal Structure

S. Ramaswami

New Approach Towards the Development of Next Generation CNC
Kamran Latif, Yusef Yusof, Qadir Bux Alias and Imran Latif

Drill String Dynamic Improving the Drilling Performance by Optimizing the Speed Limit and Study the Resonance of the Experimental Drill String System
Suriani bt Che Kar, Ibrahim Esat, Ali A.A.A. Alkhamies, Muhammad Efendy B. Mohd Fard Woo and Guillermo Schikzarian

Operation Management-Identification of New Product Development Improvement Opportunity
Tan Owee Kowang, Goh Chin Fai and Goh Sang Long

Evaluation of Resistance to Freezing and Thawing and Chloride Attack of High-Performance Concrete Applicable to Nuclear Power Plants
E.A. Seo, D.G. Kim, H.J. Lee and N.W. Yang

An Investigation of the Food Sources and Roosting Sites as Potential Factors of Hendra Virus Dispersion in South East Queensland, Australia
Jahnavee Burnham and Albert Chong

Journals by Subject

Agricultural Sciences
Animal Sciences
Applied Sciences
Business Sciences
Earth Sciences
Engineering
Information Technology
Medical Sciences
Molecular Sciences
Pharmacology
Social Sciences

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The Explants of Plantlet Induction using Auxin and Cytokinin Shortly after the Gamma Ray Irradiation and the Gripped Poly Ethylene Glycol

Ari Wijayanti and Rina Sri Iestari

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- [Agricultural Sciences](#)
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- [Applied Sciences](#)
- [Business Sciences](#)
- [Earth Sciences](#)
- [Engineering](#)
- [Information Technology](#)
- [Medical Sciences](#)
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- [Pharmacology](#)
- [Social Sciences](#)

Abstract: *Chrysanthemum* is the one of popular houseplants commodities from subtropical regions and it have already widespread in Indonesia, moreover it begins to develop from plateau until lowland. One of the problems in the lowland is a slight decreased quality of the flower. Giving improvement of the *chrysanthemum* flower quality in the medium plain, it used the suitable seeds. During this time, there is no available special variety of *chrysanthemum* flowers for medium plain and lowland, even though *chrysanthemum* flower has developed in many lowlands in Indonesia. The technology which was used for PEG osmolitikum (Poly Ethylene Glycol) in tissue culture can be used for marking the flowers which could be growth either in high temperature or low temperature. The main purpose of this research is a quantitative research investigation for the variety of explants and the optimal composition of media to stimulate the plantlet regeneration post irradiation and PEG gripped. The method that was used for this research is examining ZPT dosis through several explants which had already irradiated and gripped by gamma ray an PEG. The result of this research showed that *chrysanthemum* plantlets was stimulated with ZPT IAA+1.5 ppm 0.5 ppm BA through the comar explants to stimulate growth of shoot height, leaf, root number and root length.

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The Explants of Plantlet Induction using Auxin and Cytokinin Shortly after the Gamma Ray Irradiation and the Grippped Poly Ethylene Glycol

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Abstract: Chrysanthemum is the one of popular houseplants commodities from subtropical regions and it have already widespread in Indonesia, moreover it begins to develop from plateau until lowland. One of the problems in the lowland is a slight decreased quality of the flower. Giving improvement of the chrysanthemum flower quality in the medium plain, it used the suitable seeds. During this time, there is no available special variety of chrysanthemum flowers for medium plain and lowland, even though chrysanthemum flower has developed in many lowlands in Indonesia. The technology which was used for PEG osmolitikum (Poly Ethylene Glycol) in tissue culture can be used for marking the flowers which could be growth either in high temperature or low temperature. The main purpose of this research is a quantitative research investigation for the variety of explants and the optimal composition of matter to stimulate the plantlet regeneration post irradiation and PEG grapped. The method that was used for this research is examining ZPT dosis thought several explants which had already irradiated and grapped by gamma ray an PEG. The result of this research showed that chrysanthemum plantlets was stimulated with ZPT IAA+1.5 ppm 0.5 ppm BA through the corner explants to stimulate growth of shoot height, leaf, root number and root length.

Key words: Medium regeneration, chrysanthemum, regeneration plantlets, PEG, *in vitro*

INTRODUCTION

Chrysanthemum is origin plant from subtropical areas which it is developed in Indonesia, especially in the plateau areas. The areas under of 700 m below the plant began to developing this plant, one of them is Hargobinangun village, Pakem, Sleman. The area on the slopes of Mount Merapi with a high of 600-700 m above sea level has been designated as one of the centres of chrysanthemums by the Directorate General of Horticulture.

The approach of agro-climatic environment chrysanthemums modification needs to be processes, according to Wijayani and Muafi (2016) the day length of chrysanthemum each day which is not fixed. Based on the day length of chrysanthemum each day, chrysanthemum is the one of short day optional plant. The Critical Daylength-CDL chrysanthemum is around 13, 5-16 h

environmental modifications for instance the addition of light by using light at night needs to make on the cultivation of chrysanthemums pieces to obtain the expected plant height (vegetative phase) before flowering. The effect of length day on the physiology of flowering chrysanthemum interacts with daily temperatures frequently. On the other hand, in temperature conditions of length day around 22°C in the midday and 16°C in the night, the plants grow for plant and leaf optimally. The induction into the generative phase will occur when the temperature decreased during the day more than 18°C and the night temperature rose to less than 25°C (Harsanti and Mugiono, 2007; Fukai *et al.*, 2002).

However, this condition is extremely rare in the medium land to the plateau in Indonesia. According to Wijayani and Srilestari (2016) chrysanthemums will grow optimally if it grows in aglokilmal above 900 m sea level

and different solutions in this research, it is trying to improve the amount of tolerant varieties thought high temperatures by using *in vitro* with PEG (Poly Ethylene Glycol). The writer suspected that farmers used the seeds derived from the origin generation of trees with poor quality and moreover, it reproduces the from the origin generation of trees repeatedly from the same plant. It will reduce the quality of the plant itself (Sisworo *et al.*, 2006; Wijayani and Muafi, 2016; Sirohi and Behera, 2000; Medina *et al.*, 2004).

As the matter of fact, the main problem which is being paramount of important in this research is a complete study of various aspects of the chrysanthemum plant regarding the plantation techniques that can improve growth conditions, especially for repairing the seedlings through the chrysanthemum production tolerant for high temperatures. If the assembled chrysanthemum using PEG is successful, the special seeds for medium land can be planted in the Hargobinangun medium land. This argument can be taken to considerate to the other medium land areas of chrysanthemum plant.

MATERIALS AND METHODS

This research was conducted in tissue culture laboratory of the faculty of Agriculture UPN "Veteran" Yogyakarta in two phases. The first phase was irradiated plantlets with 25 gray of gamma rays (Poerwanto and Wijayani, 2013) which was tested through the tolerance of PEG (Poly Ethylene Glycol). In the tolerance of chrysanthemum test with good gamma irradiation have a good growth plantlet which is still green then moved into the media test. Media which was tested is adding ZPT auxin and cytokinin (0.5 ppm IAA+1.5 ppm BA, 1.0 ppm IAA+1.0 ppm BA and 1.5 ppm IAA+0.5 ppm BA) on various eksplan materials (tip, middle and base).

The implementation of this study begins with planting the plantlets from first phase results, thus, it was grown in the media with certain PEG supplemented treatments. Having four weeks after the subculture plantlets, it will show different conditions, the resistant plantlets will remain green while the plantlets that can not survive are black and die. Criteria of survival plantlets if it

subsequently stored in room with temperature incubation 24°C and intensity irradiation of 16 h per day. These treatments are done until the plants are 8 weeks old. The results were analyzed with variability for 5% significance level, then it was tested further by Duncan's multiple range test at 5% level.

RESULTS AND DISCUSSION

According to Sirohi and Behera (2000), the growth and the morphogenesis of plants through *in vitro* was controlled by the interaction and the balance between growth regulator plant which is given into the media and the growth regulator plant produced endogenously by the cultured cells.

Table 1 shows the influence of auxin in the observed parameters, the high of cells (5.1 cm) and the number of leaves (20 pieces) but the lowest percentage of their lives is around 60%. Presumably, It is caused the composition of salt minerals that exist in the media are optimal enough for the growth of chrysanthemum plantlets. Nitrogen that plants needed in large quantities is stimulated the growth of the plants, medium MS reaching 840.6763 mg/L.

The existing of the organic carbon is suspected that it will increase the activity of cell division under the apical meristems which will be followed by the stage of enlargement cell and elongation cell. The additional size will improve the high of shoots. On the other sides, the growth regulator of auxin at 1.5 ppm will be combined with very high benzyl adenine 0.5 ppm, it will being a trigger role in the growth of shoots. The auxin in stimulating the growth of shoots is mainly for arranging cell division and morphogenesis (Harsanti and Mugiono, 2007). The auxin either single factor or combination with cytokines in tissue culture has play a role in inducing and stacked of shoots (Fig. 1). Fukai *et al.* (2002) thought that in the callus roots and shoots tissue can be formed completely on their own at once without a vascular connection between them.

Figure 1 shows the addition of 1.5 ppm IAA and 0.5 ppm benzyl adenine can improve the number of shoots. It means that the interaction and the balancing between plant growth regulator that is given in the media

Table 1: The average percentage of live explants, the height of shoot and the number of leaves chrysanthemum plantlets which grown on medium regeneration with PGR after the irradiated gamma ray and the PEG grouped

Treatment	Percentage of live explants (%)	Shoot height (cm)	Number of leaves
T1 = 0.5 ppm IAA+1.5 ppm BA/tip explants	96.59 ^a	2.5 ^a	5 ^a
T2 = 0.5 ppm IAA+1.5 ppm BA/middle explants	95.15 ^a	2.0 ^a	9 ^a
T3 = 0.5 ppm IAA+1.5 ppm BA/base explants	90.60 ^a	2.2 ^a	11 ^b
T4 = 1.0 ppm IAA+1.0 ppm BA/tip explants	95.85 ^a	1.8 ^a	6 ^a
T5 = 1.0 ppm IAA+1.0 ppm BA/middle explants	86.75 ^a	3.0 ^a	6 ^a
T6 = 1.0 ppm IAA+1.0 ppm BA/base explants	80.59 ^a	2.3 ^a	10 ^a
T7 = 1.5 ppm IAA+0.5 ppm BA/tip explants	70.23 ^b	2.0 ^a	9 ^a
T8 = 1.5 ppm IAA+0.5 ppm BA/middle explants	65.10 ^b	1.8 ^a	15 ^a
T9 = 1.5 ppm IAA+0.5 ppm BA/base explants	60.00 ^b	5.1 ^a	20 ^a

The average treatments followed by the same letter show no significant difference in the level of real UJBD 5%

Table 2: The average number of roots and the length of root callus resulted from gamma ray irradiation which growth on regeneration media

Treatment	Number of roots	Length of roots (cm)
T1 = 0.5 ppm IAA+1.5 ppm BA/tip explants	3.69 ^a	1.35 ^a
T2 = 0.5 ppm IAA+1.5 ppm BA/middle explants	5.89 ^a	2.50 ^a
T3 = 0.5 ppm IAA+1.5 ppm BA/base explants	15.33 ^b	3.33 ^b
T4 = 1.0 ppm IAA+1.0 ppm BA/tip explants	11.12 ^b	1.56 ^a
T5 = 1.0 ppm IAA+1.0 ppm BA/middle explants	8.99 ^a	2.33 ^a
T6 = 1.0 ppm IAA+1.0 ppm BA/base explants	22.11 ^b	4.68 ^b
T7 = 1.5 ppm IAA+0.5 ppm BA/tip explants	18.59 ^b	4.25 ^a
T8 = 1.5 ppm IAA+0.5 ppm BA/middle explants	21.60 ^b	4.65 ^a
T9 = 1.5 ppm IAA+0.5 ppm BA/base explants	42.21 ^c	4.20 ^a

The average treatment which was followed by the same letter explained that it has not significant difference in the level of UJBD 5%



Fig. 1: The development of the base plantlets on media regeneration T9 = 1.5 ppm IAA+0.5 ppm BA

endogenously. One of the auxin roles in the process of issue culture is inducing adventitious roots on explants. The number of roots is paramount of imperative for the growth of explants *in vitro*. The higher total root and the longer then it will be great for the nutrients absorption from the media. This is because the higher root and the longer root will wider the nutrient absorption of in the root media (Sirohi and Behera, 2000). In the experiment of

formed. This was similar with the opinion that auxin (IAA) was a play rules also it had rules on the length of roots in tissue culture. Instead the cytokinins needed in small amounts thus, perhaps, the requirement cytokinin purpose for the elongation of root elongation, it have been acquired by endogenous cytokines. This is same with the opinion by Fukai *et al.* (2002) that the used of cytokines in the little amount can help in the growth of roots, while the roots that have formed will synthesize with endogenous cytokines. The use of Kinetin and IAA as a growth chrysanthemum plant shoots and roots promoters from irradiated can save energy resources and the other natural resources because the endurant examining time is faster than the conventional way, growth tunas and cells regeneration. Presumably, the IAA (auxin) caused the wall cell sagged, then, the epidermal cells elongated rapidly and the subepidermal cells attached widely, finally the roots will grow longer. The longer root and the higher roots chrysanthemum seedlings will assist in the absorption of nutrients, thus it affected on the growth plant in the head section that will develop optimally too.

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