ISBN: 978-602-9042-11-5

PROCEEDINGS

Udayana University
Press

2nd International Conference on Biosciences and Biotechnology

PAVE THE WAY TO A BETTER LIFE

Udayana University, Bali, Indonesia| 23-24 September 2010

EDITORS:

Yan Ramona (Udayana University)
Yenni Ciawi (Udayana University)
Made Pharmawati (Udayana University)
Anom Sutrisna Wijaya (Udayana University)
Nyoman Sri Budayanti (Udayana University)
Wardhana Suryapratama (Soedirman University)
Edy Kurnianto (Diponegoro University)
I Nyoman Sutarpa Sutama (Udayana University)
Ida Bagus Wayan Gunam (Udayana University)
I Made Mastika (Udayana University)
A.A. Kartini (Udayana University)
I Nyoman Sumerta Miwada (Udayana University)
I Gede Suranjaya (Udayana University)
I Gusti Putu Nugraha Yasa (Udayana University)

ISBN: 978-602-9042-11-5

PROCEEDINGS

Udayana University
Press

2nd International Conference on Biosciences and Biotechnology

PAVE THE WAY TO A BETTER LIFE

Udayana University, Bali, Indonesia
23-24 September 2010

EDITORS:

Yan Ramona (Udayana University)
Yenni Ciawi (Udayana University)
Made Pharmawati (Udayana University)
Anom Sutrisna Wijaya (Udayana University)
Nyoman Sri Budayanti (Udayana University)
Wardhana Suryapratama (Soedirman University)
Edy Kurnianto (Diponegoro University)
I Nyoman Sutarpa Sutama (Udayana University)
Ida Bagus Wayan Gunam (Udayana University)
I Made Mastika (Udayana University)
A.A. Kartini (Udayana University)
I Nyoman Sumerta Miwada (Udayana University)
I Gede Suranjaya (Udayana University)
I Gusti Putu Nugraha Yasa (Udayana University)

Preface

This proceeding is a compilation of scientific papers presented in the 2nd International Seminar on Biosciences and Biotechnology: "**Pave the Way to A Better Life**" held at the University of Udayana on 23rd – 24th 2010. It includes papers (for oral and poster presentations) presented by Keynote speakers, Invited speakers, and active participants.

This conference was designed in order to gather scientists, engineers, practitioners, and industries in Biological related disciplines, so that they could discuss and share their expertise in the fields of Biosciences and Biotechnology related issues. From this intense discussion, it was expected that some brilliant ideas to be used to improve the quality of human life could be formulated, so that it was in line with the theme of the conference: "**Biosciences and Biotechnology pave the way to a better life**".

This 2nd International conference was held in relation to the Udayana University Anniversary and is expected to be held yearly, so that this event becomes the icon of the Udayana University in the future. The conference consisted of 8 plenary presentations delivered by keynote and invited speakers with International reputations from Japan, Australia, and Indonesia, covering general aspects of Biosciences and Biotechnology. Besides this plenary sessions, we also had four satellite symposia, covering areas of **health, agricultural technology and food science, agriculture**, and **biodiversity and environment**. Totally, 175 contribution papers (oral and poster presentation) were presented in this conference and they were distributed according to the areas mentioned above. The efforts of the presenters to prepare their contribution papers for this conference are highly appreciated.

This Conference was financially supported by the Rector of Udayana University through the program of Vice Rector I (Vice Rector for Academic Affair) and some sponsors (Monsanto and Kanisius press). Therefore, in this occasion, on behalf of the committee, I would like to acknowledge their financial support.

My thanks should also go to all people who were involved in the committee of the conference. Without their hard working and efforts, I am afraid would not be able to make this event to happen.

Last but not least, I hope you all enjoyed your time in Bali, not only at the venue of the conference, but also enjoyed the beauty of Bali and the friendliness of the people, so that you all brought home some unforgettable memories about the island of Bali. See you again here next year.

Chairman of the Organizing Committee

Drs. Yan Ramona, M.App.Sc., Ph.D.

Forewords-Rector of Udayana University

Dear Distinguished guests, Invited speakers, and all other participants

This second International Conference on Biosciences and Biotechnology with the theme of Bioscience and Biotechnology pave the way to a better life is a continuation of the first International conference successfully held last year, in relation of the Udayana University Anniversary. The main aim of this conference is to gather scientists from all over the world in a venue to share their expertise in Biosciences and Biotechnology and build scientific network, so that they can develop Biosciences and Biotechnology-based methods for improving the quality of human life in the future.

In this opportunity, on behalf of the University, I welcome you all to Bali. Bali is well known as a favorite tourist destination in the world. Recently, it is also a favorite site for holding International events, such as International Conference. When people hear Bali as a site of an International conference, a lot of them will be interested to attend the event. By attending such an event in Bali, they can do two things at once. They can present scientific papers and share their expertise with other scientists known to have International reputation, and at the same time they can also enjoy the beauty of the Bali Island and the culture of Bali which is considered to be unique by foreign tourists.

Here, I would also like to acknowledge the National and International invited speakers for their willingness to come miles away to Bali and present their high standard papers. I understand that you all spend much time for this conference, and therefore I must give high appreciation on all of those effort and dedication.

I hope this International Conference become an annual agenda of Udayana University and become an ideal forum for communication and sharing ideas as well as experience in Biosciences and Biotechnology-related disciplines in the future. I also hope that this forum can serve as a forum for promoting advanced Biosciences and Biotechnology with regard to economic growth and social welfare.

Finally, I wish you most successful conference and hope that it may provide new ideas and strategies for the application of Biosciences and Biotechnology in the industries.

Rector of Udayana University,
Prof. Dr. dr. I Made Bakta, Sp.Pd (K).

EMBRYOGENIC CALLUS INDUCTION FROM MALE INFLORESCENCE OF LOCAL BANANA CULTIVARS WITH A VIEW TO PRODUCE *FUSARIUM* WILT RESISTANT PLANT *VIA IN VITRO* SELECTION [2]

Sugiyono[3], Alice Yuniaty[2,4] and Lucky Prayoga[2]
[2] Faculty of Biology, Jenderal Soedirman University, Purwokerto,Central Java, Indonesia.
[3] Faculty of Agriculture, Tadulako University, Palu Central Sulawesi, Indonesia

ABSTRACT

This I-MHERE funded research is the first step of a research program to produce *Fusarium* wilt resistant of local banana cultivars using in vitro selection approach. The objectives of this first research step are to produce sufficient material for in vitro selection. The specific objectives of this experiment was to study the influence of auxin (2,4-D and IAA) on embryogenic callus formation from male inflorescence of four local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning). An experimental research method on a *split-split plot design* has been used. The main plot was local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning), the sub plot was the kind of auxin (2,4-D, IAA), and the sub-sub plot was auxin concentration (0; 5; 10; 15 µM). All treatment combinations were replicated 3 times. The nutrient medium used was Murashige and Skoog (MS-1962) supplemented with 7.5 µM BAP and solidified with 0.8% agar. The cultures were kept in dark condition at 24 °C for six weeks. The parameters measured include the percentage of callus formation, callus formation time, and the type of callus formed. The research results showed that male inflorescence can be used as explants for mass production of embryogenic calli of 4 local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning). "Raja" cultivar was found to be the most responsive cultivar leading to the highest percentage of callus formation and short callus formation time. In addition, it was also found that no significance difference between 2,4-D and IAA on both callus formation time and the percentage explants which produced callus, however 2,4-D resulted in better embryogenic calli formation than IAA. Callus formation was controlled by the concentration of auxin applied. The higher the concentration applied, the higher the percentage of explants which produced callus.

Keywords: embryogenic callus, 2,4-D, IAA, Banana

INTRODUCTION

Indonesia is one of most important banana genetic variance centres in the world. Indonesian banana production has increased in the last 10 years from 3.3 million tones in 1999 to 6.0 million tones in 2008. However, its producing area continuously decreases, especially in Sumatra, Sulawesi, Central and east Java (Laporan Akhir Riset Unggulan Strategis Nasional 2003; Dimyati, 2004). The decrease in banana plantation area is mainly caused by pest and disease such as Panama (*Fusarium* wilt), blood, Moko, sigatoka, BBTV, CMV, stem borer and nematode (Dimyati, 2004).

Fusarium wilt disease caused by *Fusarium oxysporum* Schlecht sp cubense (Panama disease) is one of the most dangerous banana diseases. This soil-borne disease can attack banana at all banana growth phase, from seedling to adult plant, and also easily distributed (Cahyono, 1995; Balai Penelitian Tananan Buah, 2004; Purwati *et al*., 2007). Today those to pathogens have spreaded to almost all banana plantations in Indonesia, which resulted in the destruction of almost 8 million clump of banana in just 5 years period (Companioni *et al*., 2003; Widodo *et al*., 2003; Balai Penelitian Tanaman Buah, 2004; Purwati *et al*., 2007)).

Control of *Fusarium* wilt disease is extremely difficult since this pathogen form chlamidospora which able to live in the soil for a long period of time (Widodo, *et al.*, 2003). Furthermore, chemical control of this pathogen is not economically feasible. Therefore efforts to produce *Fusarium* resistance plants are paramount (Companioni *et al.*, 2003; Purwati *et al.*, 2007).

Somaclonal variation and genetic transformation are two available approaches which can be used to speed up banana improvement program. Callus culture is one of the most important steps in the induction of somaclonal variation and genetic transformation (Da Silva Conceicao *et al.*, 1998). Callus culture includes induction and culture of callus *in vitro* with a view to improved plant quality or to obtain plant secondary metabolic product (Adkins *et al*, 1990). The advantage of callus culture is the simplicity in controlling its environment both physically and chemically (Allan, 1991). It can be expected that from this type of culture genetic variant(s) can be obtained trough *somaclonal variation*, or an *in vitro* selection can be carried out to produce a desired character (Adkins, *et al*, 1990). The success of a callus culture depends on the induction stage.

With a view to produce banana embryogenic callus, various explants can be used such as immature male inflorescence (Escalant *et al.*, 1994; Ganapathi *et al.*, 1999; Gomez Kosky *et al.*, 2002; Khalil *et al*, 2002; Sidha *et al.*, 2007; Wirakarnain *et al.*, 2008), young leaves (Da Silva Conceicao *et al.*, 1998), or shoot (Srangsam and Kanchanapoom, 2003; Ramírez-Villalobos and de García, 2008).

Moreover, somaclonal variation to produce *Fusarium* wilt resistance banana can be induced by irradiation (Kosmiatin *et al.*, 2006; Zarmiyeni, 2007), *in vitro* selection with toxin such as fusaric acid ($C_{10}H_{13}O_2N$) (Matsumoto *et al.*, 1995;; Purwati *et al.*, 2007; Zarmiyeni, 2007;) or *Fusarium* culture filtrate (Lestari, 2006).

MATERIALS AND METHODS

The materials used are immature male inflorescence of 4 local banana cultivar (Raja, Ambon, Ambon Nangka, Kapok Kuning), Murashige and Skoog (MS-1962) medium , 2,4 Dichlorophenoxy Acetic Acid (2,4-D), Indole-3-aceticacid (IAA), 6-Benzylaminopurine (BAP), *casein hydrolyzate*, sucrose, agar, $HgCl_2$ 0,2%, *sterril destilate water* (SDW), ethanol 70 % and 96%.

An experimental research method on a *split-split plot design* has been used. The main plot was local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning), the sub plot was the kind of auxin (2,4-D, IAA), and the sub-sub plot was auxin concentration (0; 5; 10; 15 µM). All treatment combinations were replicated 3 times. The nutrient medium used was Murashige and Skoog (MS-1962) supplemented with 7.5 µM BAP and solidified with 0.8% agar. The cultures were kept in dark condition at 24 °C for six weeks. The parameters measured include the percentage of callus formation, callus formation time, and the type of callus formed.

RESULTS AND DISCUSSION

After six weeks of culture under dark conditions, it was observed that most explants were able to dedifferentiate to produce callus/calli (Figure 1). Callus emerged from the surface of explants as early as 17 days after culture. The time needed for callus formation was cultivar dependence. It was found that in general the fastest callus formation was observed in "Ambon Nangka" cultivar (Figure 2). Furthermore, the percentage of explants forming callus ranged from 56.77% to 92.71%. It was also found that "Raja" cultivar had the highest percentage of callus formation, in contrast "Kapok kuning" cultivar produced the lowest percentage of callus formation (Figure 3).

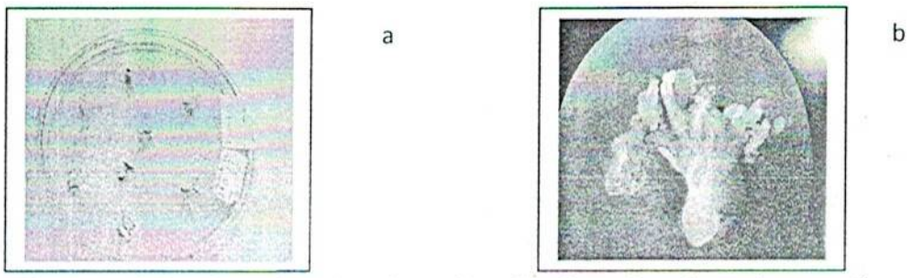

Figure 1. Callus formation: a) explants; b) callus formed on the explants surface

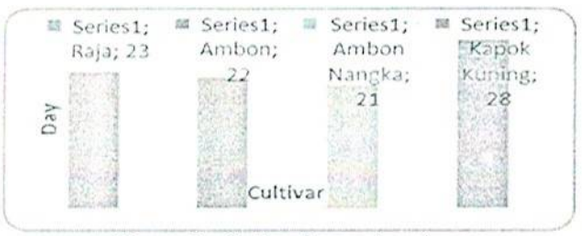

Figure 2. Callus formation time

Figure 3. The percentage of explants forming callus

It was also found that both auxins (2,4-D and IAA) can be used to induced callus formation from immature male inflorescence of banana with no significance difference on both callus formation time and the percentage explants which produced callus. However, callus formation was controlled by the concentration of auxin applied. The higher the concentration applied the higher the percentage of explants which produced callus (Figure 4). In addition, no significance difference was observed on the effect of auxin concentration on callus emergence.

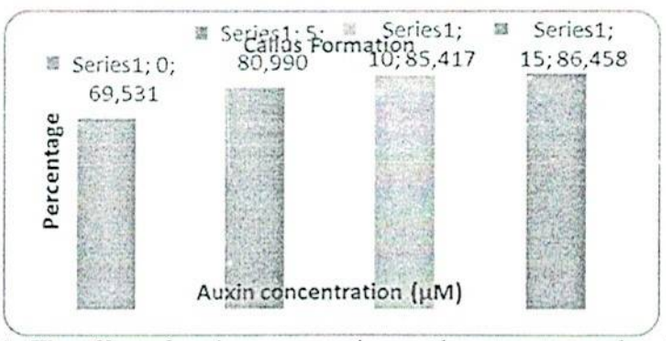

Figure 4. The effect of auxin concentration on the percentage of explants forming callus

Further examination on the type of callus formed, it was found that all three types of callus i.e. embryogenic, proliferative, and senescence were formed (Figure 5). Further data analysis showed that 2,4-D application resulted in higher percentage of embryogenic callus formation. In contrast, IAA application resulted in high percentage of senescence callus formation (Figure 6). However, the high percentage of proliferative and senescence callus formation was not expected.

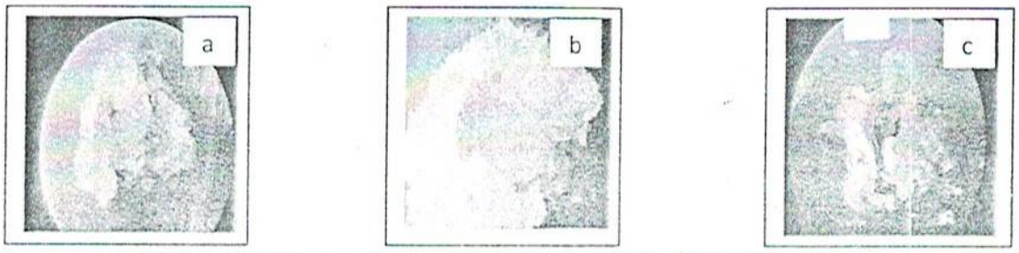

Figure 5. Banana callus type: a) embryogenic; b) proliferative; c) senescence

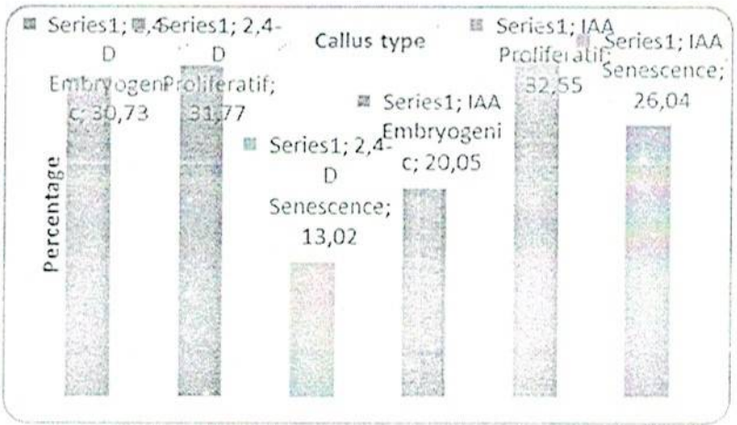

Figure 6. The effect of auxin concentration on the type of callus formed

The successful callus induction from immature male inflorescence of five local banana cultivar implied that banana male inflorescence can be used as explants for mass production of embryogenic calli. Callus induction from immature male inflorescence of banana has also been reported by Escalant *et al.*, (1994); Ganapathi *et al.*, (1999); Gomez Kosky *et al.*, (2002); Khalil *et al*, (2002); Sidha *et al.*, (2007); and Wirakarnain *et al.*, (2008).

Banana callus induction was carried out on Murashige and Skoog media supplemented with auxin and cytokinin. The used of MS medium, auxin and cytokinin for banana callus induction from immature male inflorescence have been reported elsewhere (Da Silva Conceicao *et al.*, 1998; Ganapathi *et al.*, 1999; Gomez Kosky *et al.*, 2002; Srangsam and Kanchanapoom, 2003; and Wirakarnain *et al.*, 2008). The used of MS media resulted in better embryogenic callus formation that that of White medium (Ganapathi *et al.*, 1999).

In addition, the least expected high percentage of proliferative and senescence callus formation might have been caused by high cytokinin application in callus induction medium. 7.5 μM 6-benzylaminopurine (BAP) was supplemented in all media used. High cytokinin concentration will induce high cell multiplication leading to proliferative and senescence callus formation.

Callus morphology in callus culture can be classified into 3 types (Kesee *et al*, 1991):

a. Developmental/embryogenic callus, i.e. callus which capable of developing into somatic embryogenesis or somatic organogenesis. This type of callus is characterised by the formation of chlorophyll, green in colour, compact and fast growing.
b. Proliferative callus i.e. callus which has a very high multiplication rate leading to the formation of a massive amount of cells but very small in size. This type of callus has very limited cytoplasm which also incapable of regenerating.
c. Senescence callus i.e. a very slow growing callus and shows no symptom of development. This type of callus is characterised by the absence of chlorophyll, brownish in colour, watery, and polyhedral cell form.`

CONCLUSIONS

It can be concluded that male inflorescence can be used as explants for mass production of embryogenic calli of 4 local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning). "Raja" cultivar was found to be the most responsive cultivar leading to the highest percentage of callus formation and short callus formation time. In addition, it was also found that no significance difference between 2,4-D and IAA on both callus formation time and the percentage explants which produced callus, however 2,4-D resulted in better embryogenic calli formation than IAA. Callus formation was controlled by the concentration of auxin applied. The higher the concentration applied, the higher the percentage of explants which produced callus.

ACKNOWLEDMENTS

This research was funded by I-MHERE project of Jenderal Soedirman University, which therefore the author thank you for. Great appreciation was also dedicated to all students involved in this project (Dwi Indah Purnamasari, Berti Pratiwi, Tina Agustina, Hari Hartiko, Anis Anov I., Diah Wulan Sari, Putri Cindiarum, Esti Rahma Astuti, Uud Pandu Suandhana) for their hard work and dedication.

REFERENCES

Adkins SW, Shiraishi T, McComb JA, Ratanopol S, Kupkanchanakul T, Amstrong LJ, Schultz AL. 1990. Somaclonal variation in rice–submergence tolerance and other agronomic characters. ***Physiologia Plantarum* 80: 647 – 654.**

Allan E. 1991. Plant Cell Culture. In Stafford, A and Warren, G. (eds.), 1991. *Plant Cell and Tissue Culture*. Open University Press. Buckingham.

Balai Penelitian Tanaman Buah. 2004. Eradikasi Tanaman Pisang Terserang Penyakit Layu. Badan Penelitian dan Pengembangan Pertanian, Departemen Pertanian.

Cahyono B. 1995. Pisang, Budidaya dan Analisis Usaha Tani. Kanisius, Yogyakarta

Companioni B, Arzola M, Rodr´ıguez Y, Mosqueda M, P´erez MC, Borr´as O, Lorenzo JC, Santos R, 2003. Use Of Culture-Derived *Fusarium* Oxysporum F. Sp. Cubense, Race 1 filtrates For Rapid And Non-Destructive *In vitro* Differentiation Between Resistant And Susceptible Clones Of field-Grown Banana. **Euphytica 130: 341–347.**

da Silva Conceicao A, Matsumoto K, Bakry F, Berno Souza RB. 1998. Plant Regeneration From Long-Term Callus Culture Of Aaa-Group Dessert Banana. **Pesquisa Agropecuaria Brasileira, 33 (8) : 1291-1296.**

Dimyati A. 2004. Progress report of pests and diseases research on banana in Indonesia. *In*. Molina A.B., Eusebio, J.E., Roa V.N., Van Den Bergh I. and Maghuyop M.A.G.,

Borromeo K.H. (eds). Advancing banana and plantain R&D in Asia and the Pacific - Vol. 12. **Proceedings of the 2st BAPNET Steering Committee meeting**. Jakarta

Escalant JV, Teisson C, Cote F. 1994. Amplified Somatic Embryogenesis from Male Flowers Of Triploid Banana And Plantain Cultivars (*Musa Spp.*) **In Vitro Cell. Dev. Biol**. 30: pp 181-186

G´omez Kosky R, De Feria Silva M, Perez LP, Terrence BG, Francisco M, Reyes Vega M, Chavez Milian M, Quiala Mendoza Elisa M, 2002. Somatic embryogenesis of the banana hybrid cultivar FHIA-18 (AAAB) in liquid medium and scaled-up in a bioreactor. **Plant cell, tissue and organ Culture 68 (01):pp. 21-26**

Ganapathi TR, Suprasanna P, Bapat VA, Kulkarni VM and Rao PS. 1999. Somatic Embryogenesis And Plant Regeneration From Male Flower Buds In Banana Http://Www.Ias.Ac.In/Currsci/May10/Articles18.Htm

Kesee RJ, Rupert GE, Carter Jr. 1991. Investigations of Proliferative and Senescent Callus of Soybean. **Physiologia Plantarum, Vol. 81 (4): 555-557.**

Khalil SM, Cheah KT, Perez EA, Gaskill DA, Hu JS. 2002. Regeneration of banana (*Musa spp.* AAB cv. Dwarf Brazilian) via secondary somatic embryogenesis. **Plant Cell Reports, 20:1128–1134.**

Kosmiatin M, Mariska I, Roostika I dan Gati E. 2006. Pembentukan Pisang Ambon Toleran terhadap Penyakit Layu *Fusarium* melalui Variasi Somaklonal. **Zuriat 17 (1) :16-24**

Laporan Akhir Riset Unggulan Strategis Nasional 2003. Pengembangan Buah-Buahan Unggulan Indonesia http://pkbt.ipb.ac.id/pages/exsum/2003-nenas.pdf).

Lestari EG. 2006. Review: In Vitro Selection and Somaclonal Variation for Biotic and Abiotic Stress Tolerance. **Biodiversitas, 7 (3): 297-301.**

Matsumoto K, Barbosa ML, Souza LAC, Teixeira JB. 1995. Race 1 *Fusarium* wilt tolerance on banana plants selected by fusaric acid. **Euphytica 84:67-71.**

Purwati RD, Setyo-Budi U, Sudarsono. 2007. Penggunaan Asam Fusarat Dalam Seleksi *In vitro* Untuk Resistensi Abaka Terhadap *Fusarium* oxysporum F.Sp. Cubense, **Jurnal Littri Vol.13 (2): 64 – 72.**

Ramírez-Villalobos M and de García E. 2008. Obtainment of embryogenic cell suspensions from scalps of the banana CIEN-BTA-03 (Musa sp., AAAA) and regeneration of the plants. **Electronic Journal of Biotechnology Vol.11(5), Special Issue.**

Sidha M, Suprasanna P, Bapat VA, Kulkarni UG, Shinde BN. 2007. Developing Somatic Embryogenic Culture System And Plant Regeneration In Banana. **FOUNDER'S DAY SPECIAL ISSUE: Issue No 285,October 2007.**

Srangsam A and Kanchanapoom K. 2003. Thidiazuron induced plant regeneration in callus culture of triploid banana (Musa sp.) 'Gros Michel', AAA group. **Songklanakarin J. Sci. Technol., 2003, 25(6) : 689-696.**

Widodo S, Sumardiyono C, Hadisutrisno B. 2003. Pengimbasan Ketahanan Pisang Terhadap Penyakit Layu *Fusarium* dengan *Burkholderia Cepacia*. **Agrosains 5 (2): 72-79.**

Wirakarnain S, Hossain ABMS, Chandran S, 2008. Plantlet Production through Development of Competent Multiple Meristem Cultures from Male Inflorescence of Banana, Musa acuminta cv. 'Pisang Mas' (AA). **American Journal of Biochemistry and Biotechnology 4 (4): 325-328.**

Zarmiyeni, Kasli M, Kasim, Suliansyah I, Nasir N, Ishak. 2007. Seleksi In Vitro untuk Resistensi Mutan Pisang Barangan Terhadap F. Oxysporum F.Sp. Cubense Menggunakan Asam Fusarat Sebagai Agens Penyeleksi. **Jurnal Ilmu-Ilmu Pertanian Indonesia. Edisi Khusus, No. 3, Hal. 427 – 431.**

REGISTRATION FORM

International Seminar On Biosciences And Biotechnology: 'Pave The Way To A Better Life'

Universitas Udayana

23 – 24 september 2010

Please send back the filled form to: astarini@biologi.unud.ac.id or idaastarini@yahoo.com

Title : (Prof/Dr/Mr./MS)	: Ph.D.
Name	: Sugiyono
Institution	: Jenderal Soedirman University
Address	: Rektorat Unsoed Lt 1 Jl. HR Boenyamin 708
City	: Purwokerto, Central Java
Country	: Indonesia.
Post code	: 53122
Tel office	: +62-281-628710
Mobile	: +6281327396412.
email	: gieks_sugiyono@hotmail.com.

Paper title : (please indicate spoken/oral or poster presentation)

EMBRYOGENIC CALLUS INDUCTION FROM MALE INFLORESCENCE OF LOCAL BANANA CULTIVARS WITH A VIEW TO PRODUCE FUSARIUM WILT RESISTANT PLANT *VIA IN VITRO* SELECTION *(submitted for spoken/oral presentation)*

Abstract : (in English, max 350 words, Times New Roman, single space)

CERTIFICATE OF PARTICIPATION

This is to certify that

Sugiyono

has participated as

PRESENTER

in 2nd International Conference on **Bioscience and Biotechnology**
PAVE THE WAY FOR A BETTER LIFE
23 - 24 September 2010
Bali, Indonesia
organized by Udayana University

KEMENTERIAN PENDIDIKAN NASIONAL
UNIVERSITAS UDAYANA
REKTOR

Prof. Dr. dr. I Made Bakta, Sp.PD (KH OM)
Rector of Udayana University

UNIVERSITAS UDAYANA
INTERNATIONAL CONFERENCE ON
"BIOSCIENCES and BIOTECHNOLOGY"

Dr. Yan Ramona
Head of Organising Commitee

UNIVERSITAS UDAYANA

INTERNATIONAL SEMINAR ON BIOSCIENCES AND BIOTECHNOLOGY:

'PAVE THE WAY TO A BETTER LIFE'

23 – 24 SEPTEMBER 2010

No : 50/SI/2010

Date: 15th September 2010

Sugiyono
Jenderal Soedirman University
e-mail: gieks_sugiyono@hotmail.com

Dear Sugiyono

On behalf of the Organizing Committee of the International Conference on Biosciences and Biotechnology, we are pleased to inform you that your paper under the title " **EMBRYOGENIC CALLUS INDUCTION FROM MALE INFLORESCENCE OF LOCAL BANANA CULTIVARS WITH A VIEW TO PRODUCE FUSARIUM WILT RESISTANT PLANT *VIA IN VITRO* SELECTION** " has been accepted for Oral Presentation during the Conference, which will be held on September 23rd – 24th, 2010, in Bali-Indonesia.

The details of the congress program will be communicated to you at the earliest.

We are looking forward to meeting you in Bali at the conference.

Best regards,

Dr. Yan Ramona
Chairman/Secretary of the Organizing Committee

International Conference on Biosciences and Biotechnology 2010
Bali-Indonesia

REGISTRATION FORM

International Seminar On Biosciences And Biotechnology: 'Pave The Way To A Better Life'

Universitas Udayana

23 – 24 september 2010

Please send back the filled form to: astarini@biologi.unud.ac.id or idaastarini@yahoo.com

Title : (Prof/Dr/Mr./MS)	: Ph.D.
Name	: Sugiyono
Institution	: Jenderal Soedirman University
Address	: Rektorat Unsoed Lt 1 Jl. HR Boenyamin 708
City	: Purwokerto, Central Java
Country	: Indonesia.
Post code	: 53122
Tel office	: +62-281-628710
Mobile	: +6281327396412.
email	: gieks_sugiyono@hotmail.com.

Paper title : (please indicate spoken/oral or poster presentation)

EMBRYOGENIC CALLUS INDUCTION FROM MALE INFLORESCENCE OF LOCAL BANANA CULTIVARS WITH A VIEW TO PRODUCE FUSARIUM WILT RESISTANT PLANT *VIA IN VITRO* SELECTION *(submitted for spoken/oral presentation)*

Abstract : (in English, max 350 words, Times New Roman, single space)

EMBRYOGENIC CALLUS INDUCTION FROM MALE INFLORESCENCE OF LOCAL BANANA CULTIVARS WITH A VIEW TO PRODUCE *FUSARIUM* WILT RESISTANT PLANT *VIA IN VITRO* SELECTION [1]

Sugiyono[2], Alice Yuniaty[2,3] dan Lucky Prayoga[2]

Abstract

This I-MHERE funded research is the first step of a research program to produce *Fusarium* wilt resistant of local banana cultivars using in vitro selection approach. The objectives of this first research step are to produce sufficient material for in vitro selection. The specific objectives of this experiment was to study the influence of auxin (2,4-D and IAA) on embryogenic callus formation from male inflorescence of four local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning).

An experimental research method on a *split-split plot design* has been used. The main plot was local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning), the sub plot was the kind of auxin (2,4-D, IAA), and the sub-sub plot was auxin concentration (0; 5; 10; 15 µM). All treatment combinations were replicated 3 times. The nutrient medium used was Murashige and Skoog (MS-1962) supplemented with 7.5 µM BAP and solidified with 0.8% agar. The cultures were kept in dark condition at 24 °C for six weeks. The parameters measured include the percentage of callus formation, callus formation time, and the type of callus formed.

The research results showed that male inflorescence can be used as explants for mass production of embryogenic calli of 4 local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning). "Raja" cultivar was found to be the most responsive cultivar leading to the highest percentage of callus formation and short callus formation time. In addition, it was also found that no significance difference between 2,4-D and IAA on both callus formation time and the percentage explants which produced callus, however 2,4-D resulted in better embryogenic calli formation than IAA. Callus formation was controlled by the concentration of auxin applied. The higher the concentration applied, the higher the percentage of explants which produced callus.

Keywords: embryogenic callus, 2,4-D, IAA, Banana

INTRODUCTION

Indonesia is one of most important banana genetic variance centres in the world. Indonesian banana production has increased in the last 10 years from 3.3 million tones in 1999 to 6.0 million tones in 2008. However, its producing area continuously decreases, especially in

---

[1] Paper submitted to International Seminar On Biosciences And Biotechnology: 'Pave The Way To A Better Life' Universitas Udayana 23 – 24 September 2010

[2] Faculty of Biology, Jenderal Soedirman University, Purwokerto,Central Java, Indonesia.
[3] Faculty of Agriculture, Tadulako University, Palu Central Sulawesi, Indonesia

EMBRYOGENIC CALLUS INDUCTION FROM MALE INFLORESCENCE OF LOCAL BANANA CULTIVARS WITH A VIEW TO PRODUCE *FUSARIUM* WILT RESISTANT PLANT *VIA IN VITRO* SELECTION [1]

Sugiyono[2], Alice Yuniaty[2,3] dan Lucky Prayoga[2]

Abstract

This I-MHERE funded research is the first step of a research program to produce *Fusarium* wilt resistant of local banana cultivars using in vitro selection approach. The objectives of this first research step are to produce sufficient material for in vitro selection. The specific objectives of this experiment was to study the influence of auxin (2,4-D and IAA) on embryogenic callus formation from male inflorescence of four local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning).

An experimental research method on a *split-split plot design* has been used. The main plot was local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning), the sub plot was the kind of auxin (2,4-D, IAA), and the sub-sub plot was auxin concentration (0; 5; 10; 15 µM). All treatment combinations were replicated 3 times. The nutrient medium used was Murashige and Skoog (MS-1962) supplemented with 7.5 µM BAP and solidified with 0.8% agar. The cultures were kept in dark condition at 24 °C for six weeks. The parameters measured include the percentage of callus formation, callus formation time, and the type of callus formed.

The research results showed that male inflorescence can be used as explants for mass production of embryogenic calli of 4 local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning). "Raja" cultivar was found to be the most responsive cultivar leading to the highest percentage of callus formation and short callus formation time. In addition, it was also found that no significance difference between 2,4-D and IAA on both callus formation time and the percentage explants which produced callus, however 2,4-D resulted in better embryogenic calli formation than IAA. Callus formation was controlled by the concentration of auxin applied. The higher the concentration applied, the higher the percentage of explants which produced callus.

Keywords: embryogenic callus, 2,4-D, IAA, Banana

INTRODUCTION

Indonesia is one of most important banana genetic variance centres in the world. Indonesian banana production has increased in the last 10 years from 3.3 million tones in 1999 to 6.0 million tones in 2008. However, its producing area continuously decreases, especially in

[1] Paper submitted to International Seminar On Biosciences And Biotechnology: 'Pave The Way To A Better Life' Universitas Udayana 23 – 24 September 2010

[2] Faculty of Biology, Jenderal Soedirman University, Purwokerto,Central Java, Indonesia.
[3] Faculty of Agriculture, Tadulako University, Palu Central Sulawesi, Indonesia

EMBRYOGENIC CALLUS INDUCTION FROM MALE INFLORESCENCE OF LOCAL BANANA CULTIVARS WITH A VIEW TO PRODUCE *FUSARIUM* WILT RESISTANT PLANT *VIA IN VITRO* SELECTION [1]

Sugiyono[2], Alice Yuniaty[2,3] dan Lucky Prayoga[2]

Abstract

This I-MHERE funded research is the first step of a research program to produce *Fusarium* wilt resistant of local banana cultivars using in vitro selection approach. The objectives of this first research step are to produce sufficient material for in vitro selection. The specific objectives of this experiment was to study the influence of auxin (2,4-D and IAA) on embryogenic callus formation from male inflorescence of four local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning).

An experimental research method on a *split-split plot design* has been used. The main plot was local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning), the sub plot was the kind of auxin (2,4-D, IAA), and the sub-sub plot was auxin concentration (0; 5; 10; 15 µM). All treatment combinations were replicated 3 times. The nutrient medium used was Murashige and Skoog (MS-1962) supplemented with 7.5 µM BAP and solidified with 0.8% agar. The cultures were kept in dark condition at 24 °C for six weeks. The parameters measured include the percentage of callus formation, callus formation time, and the type of callus formed.

The research results showed that male inflorescence can be used as explants for mass production of embryogenic calli of 4 local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning). "Raja" cultivar was found to be the most responsive cultivar leading to the highest percentage of callus formation and short callus formation time. In addition, it was also found that no significance difference between 2,4-D and IAA on both callus formation time and the percentage explants which produced callus, however 2,4-D resulted in better embryogenic calli formation than IAA. Callus formation was controlled by the concentration of auxin applied. The higher the concentration applied, the higher the percentage of explants which produced callus.

Keywords: embryogenic callus, 2,4-D, IAA, Banana

INTRODUCTION

Indonesia is one of most important banana genetic variance centres in the world. Indonesian banana production has increased in the last 10 years from 3.3 million tones in 1999 to 6.0 million tones in 2008. However, its producing area continuously decreases, especially in

---

[1] Paper submitted to International Seminar On Biosciences And Biotechnology: 'Pave The Way To A Better Life' Universitas Udayana 23 – 24 September 2010

[2] Faculty of Biology, Jenderal Soedirman University, Purwokerto,Central Java, Indonesia.
[3] Faculty of Agriculture, Tadulako University, Palu Central Sulawesi, Indonesia

Sumatra, Sulawesi, Central and east Java (Laporan Akhir Riset Unggulan Strategis Nasional 2003; Dimyati, 2004). The decrease in banana plantation area is mainly caused by pest and disease such as Panama (*Fusarium* wilt), blood, Moko, sigatoka, BBTV, CMV, stem borer and nematode (Dimyati, 2004).

Fusarium wilt disease caused by *Fusarium oxysporum* Schlecht sp cubense (Panama disease) is one of the most dangerous banana diseases. This soil-borne disease can attack banana at all banana growth phase, from seedling to adult plant, and also easily distributed (Cahyono, 1995; Balai Penelitian Tanaman Buah, 2004; Purwati *et al*., 2007). Today those to pathogens have spreaded to almost all banana plantations in Indonesia, which resulted in the destruction of almost 8 million clump of banana in just 5 years period (Companioni *et al*., 2003; Widodo *et al*., 2003; Balai Penelitian Tanaman Buah, 2004; Purwati *et al*., 2007).

Control of *Fusarium* wilt disease is extremely difficult since this pathogen form chlamidospora which able to live in the soil for a long period of time (Widodo, *et al*., 2003). Furthermore, chemical control of this pathogen is not economically feasible. Therefore efforts to produce *Fusarium* resistance plants are paramount (Companioni *et al*., 2003; Purwati *et al*., 2007).

Somaclonal variation and genetic transformation are two available approaches which can be used to speed up banana improvement program. Callus culture is one of the most important steps in the induction of somaclonal variation and genetic transformation (Da Silva Conceicao *et al*., 1998). Callus culture includes induction and culture of callus *in vitro* with a view to improved plant quality or to obtain plant secondary metabolic product (Adkins *et al*, 1990). The advantage of callus culture is the simplicity in controlling its environment both physically and chemically (Allan, 1991). It can be expected that from this type of culture genetic variant(s) can be obtained trough *somaclonal variation,* or an *in vitro* selection can be carried out to produce a desired character (Adkins, *et al*, 1990). The success of a callus culture depends on the induction stage.

With a view to produce banana embryogenic callus, various explants can be used such as immature male inflorescence (Escalant *et al*., 1994; Ganapathi *et al*., 1999; Gomez Kosky *et al*., 2002; Khalil *et al*, 2002; Sidha *et al*., 2007; Wirakarnain *et al*., 2008), young leaves (Da Silva Conceicao *et al*., 1998), or shoot (Srangsam and Kanchanapoom, 2003; Ramírez-Villalobos and de García, 2008).

Moreover, somaclonal variation to produce *Fusarium* wilt resistance banana can be induced by irradiation (Kosmiatin *et al.*, 2006; Zarmiyeni, 2007), *in vitro* selection with toxin such as fusaric acid ($C_{10}H_{13}O_2N$) (Matsumoto *et al.*, 1995;; Purwati *et al.*, 2007; Zarmiyeni, 2007;) or *Fusarium* culture filtrate (Lestari, 2006).

MATERIAL AND METHODS

The materials used are immature male inflorescence of 4 local banana cultivar (Raja, Ambon, Ambon Nangka, Kapok Kuning), Murashige and Skoog (MS-1962) medium , 2,4 Dichlorophenoxy Acetic Acid (2,4-D), Indole-3-aceticacid (IAA), 6-Benzylaminopurine (BAP), *casein hydrolyzate*, sucrose, agar, $HgCl_2$ 0,2%, *sterril destilate water* (SDW), ethanol 70 % and 96%.

An experimental research method on a *split-split plot design* has been used. The main plot was local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning), the sub plot was the kind of auxin (2,4-D, IAA), and the sub-sub plot was auxin concentration (0; 5; 10; 15 µM). All treatment combinations were replicated 3 times. The nutrient medium used was Murashige and Skoog (MS-1962) supplemented with 7.5 µM BAP and solidified with 0.8% agar. The cultures were kept in dark condition at 24 ^{o}C for six weeks. The parameters measured include the percentage of callus formation, callus formation time, and the type of callus formed.

RESULTS AND DISCUSSION

After six weeks of culture under dark conditions, it was observed that most explants were able to dedifferentiate to produce callus/calli (Figure 1). Callus emerged from the surface of explants as early as 17 days after culture. The time needed for callus formation was cultivar dependence. It was found that in general the fastest callus formation was observed in "Ambon Nangka" cultivar (Figure 2). Furthermore, the percentage of explants forming callus ranged from 56.77% to 92.71%. It was also found that "Raja" cultivar had the highest percentage of callus formation, in contrast "Kapok kuning" cultivar produced the lowest percentage of callus formation (Figure 3).

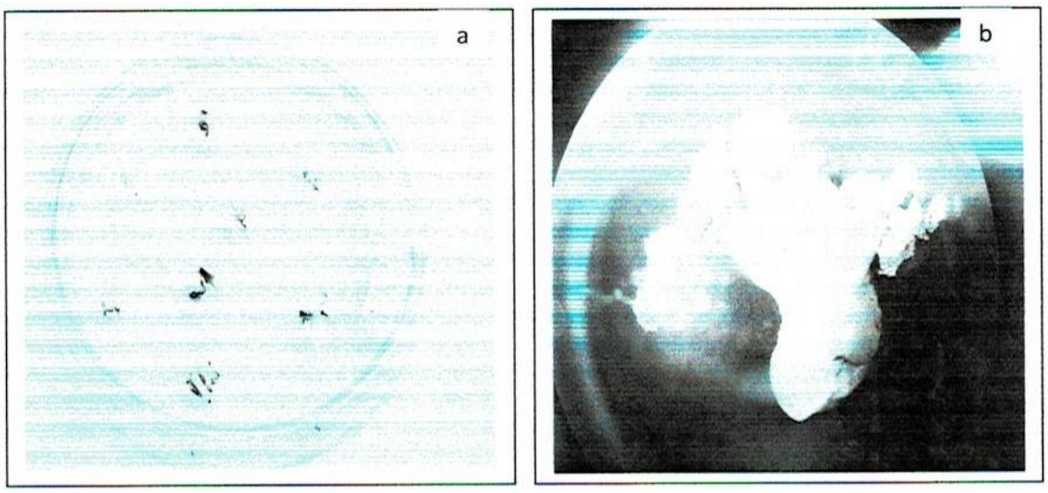

Figure 1. Callus formation: a) explants; b) callus formed on the explants surface

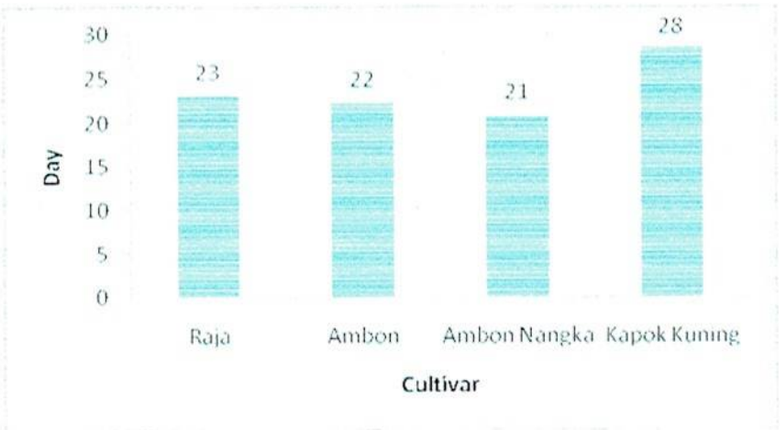

Figure 2. Callus formation time

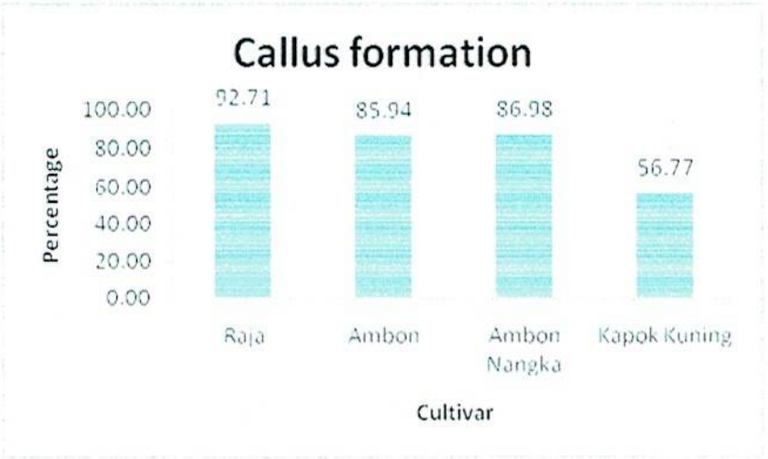

Figure 3. The percentage of explants forming callus

It was also found that both auxins (2,4-D and IAA) can be used to induced callus formation from immature male inflorescence of banana with no significance difference on both callus formation time and the percentage explants which produced callus. However, callus formation was controlled by the concentration of auxin applied. The higher the concentration applied the higher the percentage of explants which produced callus (Figure 4). In addition, no significance difference was observed on the effect of auxin concentration on callus emergence.

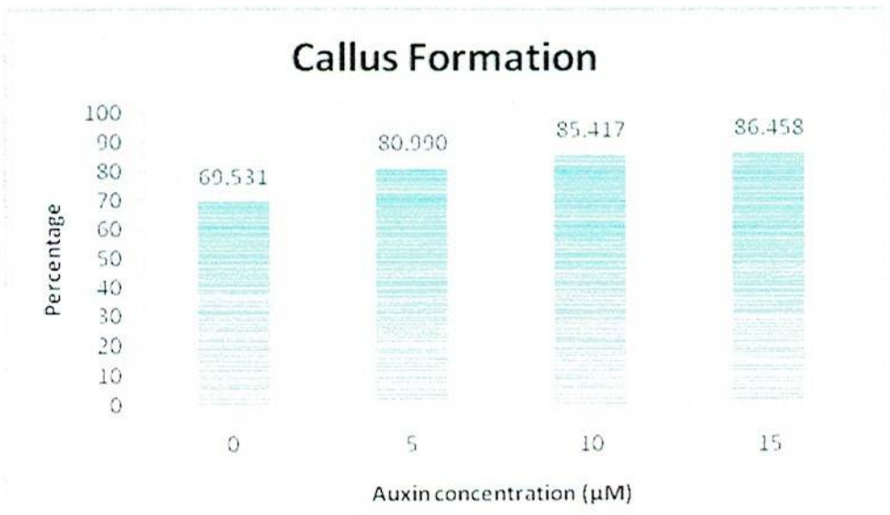

Figure 4. The effect of auxin concentration on the percentage of explants forming callus

Further examination on the type of callus formed, it was found that all three types of callus i.e. embryogenic, proliferative, and senescence were formed (Figure 5). Further data analysis showed that 2,4-D application resulted in higher percentage of embryogenic callus formation. In contrast, IAA application resulted in high percentage of senescence callus formation (Figure 6). However, the high percentage of proliferative and senescence callus formation was not expected.

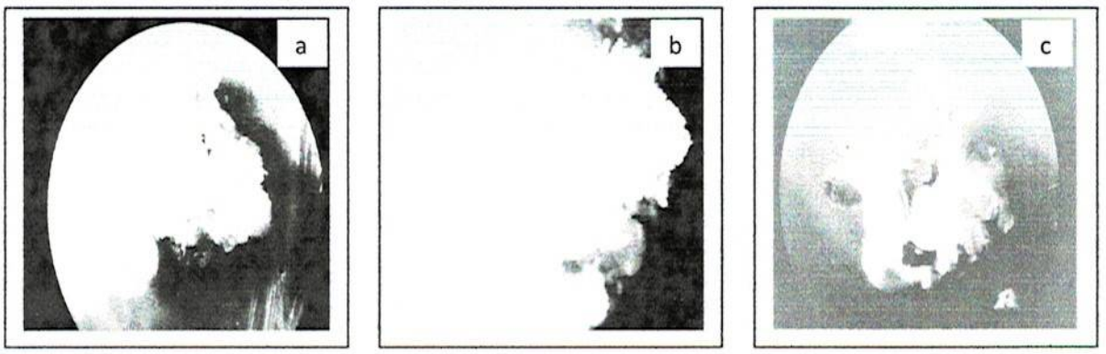

Figure 5. Banana callus type: a) embryogenic; b) proliferative; c) senescence

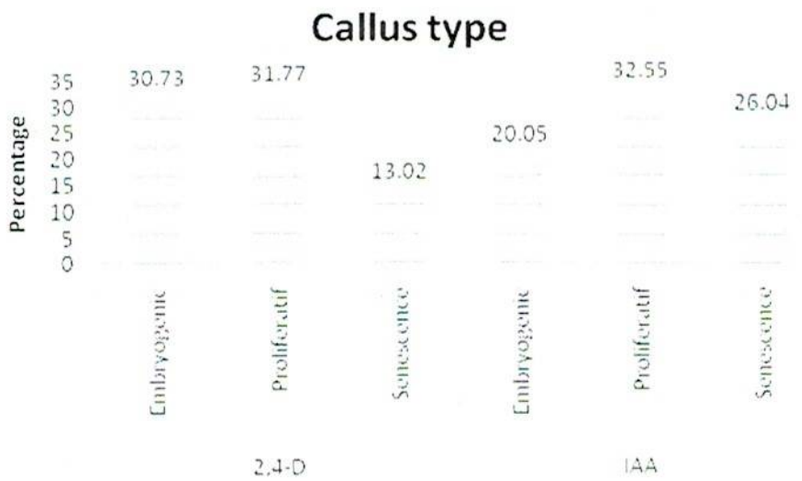

Figure 6. The effect of auxin concentration on the type of callus formed

The successful callus induction from immature male inflorescence of five local banana cultivar implied that banana male inflorescence can be used as explants for mass production of embryogenic calli. Callus induction from immature male inflorescence of banana has also been reported by Escalant *et al*., (1994); Ganapathi *et al*., (1999); Gomez Kosky *et al*., (2002); Khalil *et al*, (2002); Sidha *et al*., (2007); and Wirakarnain *et al*., (2008).

Banana callus induction was carried out on Murashige and Skoog media supplemented with auxin and cytokinin. The used of MS medium, auxin and cytokinin for banana callus induction from immature male inflorescence have been reported elsewhere (Da Silva Conceicao *et al*., 1998; Ganapathi *et al*., 1999; Gomez Kosky *et al*., 2002; Srangsam and Kanchanapoom, 2003; and Wirakarnain *et al*., 2008). The used of MS media resulted in better embryogenic callus formation that that of White medium (Ganapathi *et al*., 1999).

In addition, the least expected high percentage of proliferative and senescence callus formation might have been caused by high cytokinin application in callus induction medium. 7.5 µM 6-benzylaminopurine (BAP) was supplemented in all media used. High cytokinin concentration will induce high cell multiplication leading to proliferative and senescence callus formation.

Callus morphology in callus culture can be classified into 3 types (Kesee *et al*, 1991):

a. Developmental/embryogenic callus, i.e. callus which capable of developing into somatic embryogenesis or somatic organogenesis. This type of callus is characterised by the formation of chlorophyll, green in colour, compact and fast growing.
b. Proliferative callus i.e. callus which has a very high multiplication rate leading to the formation of a massive amount of cells but very small in size. This type of callus has very limited cytoplasm which also incapable of regenerating.
c. Senescence callus i.e. a very slow growing callus and shows no symptom of development. This type of callus is characterised by the absence of chlorophyll, brownish in colour, watery, and polyhedral cell form.`

CONCLUSSIONS

It can be concluded that male inflorescence can be used as explants for mass production of embryogenic calli of 4 local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning). "Raja" cultivar was found to be the most responsive cultivar leading to the highest percentage of callus formation and short callus formation time. In addition, it was also found that no significance difference between 2,4-D and IAA on both callus formation time and the percentage explants which produced callus, however 2,4-D resulted in better embryogenic calli formation than IAA. Callus formation was controlled by the concentration of auxin applied. The higher the concentration applied, the higher the percentage of explants which produced callus.

ACKNOWLEDMENTS

This research was funded by I-MHERE project of Jenderal Soedirman University, which therefore the author thank you for. Great appreciation was also dedicated to all students involved in this project (Dwi Indah Purnamasari, Berti Pratiwi, Tina Agustina, Hari Hartiko, Anis Anov I., Diah Wulan Sari, Putri Cindiarum, Esti Rahma Astuti, Uud Pandu Suandhana) for their hard work and dedication.

REFERENCES

Adkins, S.W., Shiraishi, T., McComb, J.A., Ratanopol, S., Kupkanchanakul, T., Amstrong, L.J., Schultz, A.L., 1990. Somaclonal variation in rice – submergence tolerance and other agronomic characters. ***Physiologia Plantarum* 80: 647 – 654**.

Allan, E., 1991. Plant Cell Culture. In Stafford, A and Warren, G. (eds.), 1991. *Plant Cell and Tissue Culture*. Open University Press. Buckingham.

Balai Penelitian Tanaman Buah, 2004. Eradikasi Tanaman Pisang Terserang Penyakit Layu. Badan Penelitian dan Pengembangan Pertanian, Departemen Pertanian.

Cahyono, B. 1995. Pisang, Budidaya dan Analisis Usaha Tani. Kanisius, Yogyakarta

Companioni, B., M. Arzola, Y. Rodr´ıguez, M. Mosqueda, M.C. P´erez, O. Borr´as, J. C. Lorenzo, R. Santos, 2003. Use Of Culture-Derived *Fusarium* Oxysporum F. Sp. Cubense, Race 1 filtrates For Rapid And Non-Destructive *In vitro* Differentiation Between Resistant And Susceptible Clones Of field-Grown Banana. **Euphytica 130: 341–347**.

Da Silva Conceicao A., K. Matsumoto, F. Bakry, R.B, Berno Souza, 1998. Plant Regeneration From Long-Term Callus Culture Of Aaa-Group Dessert Banana. **Pesquisa Agropecuaria Brasileira, 33 (8) : 1291-1296.**

Dimyati, A., 2004. Progress report of pests and diseases research on banana in Indonesia. *In.* Molina A.B., Eusebio, J.E., Roa V.N., Van Den Bergh I. and Maghuyop M.A.G., Borromeo K.H. (eds). Advancing banana and plantain R&D in Asia and the Pacific - Vol. 12. **Proceedings of the 2st BAPNET Steering Committee meeting**. Jakarta

Escalant, J-V., C. Teisson, F. Cote , 1994. Amplified Somatic Embryogenesis From Male Flowers Of Triploid Banana And Plantain Cultivars (*Musa Spp.*) **In Vitro Cell. Dev. Biol**. 30: pp 181-186

G´omez Kosky, R., M. De Feria Silva, L. Posada Perez, G Terrence, B. Martinez Francisco, M. Reyes Vega, M. Chavez Milian, E. Quiala Mendoza Elisa, 2002. Somatic embryogenesis of the banana hybrid cultivar FHIA-18 (AAAB) in liquid medium and scaled-up in a bioreactor. **Plant cell, tissue and organ Culture 68 (01):pp. 21-26**

Ganapathi, T. R., P. Suprasanna, V. A. Bapat, V. M. Kulkarni And P. S. Rao, 1999. Somatic Embryogenesis And Plant Regeneration From Male Flower Buds In Banana Http://Www.Ias.Ac.In/Currsci/May10/Articles18.Htm

Kesee, R.J., Rupert, G.E. Carter Jr., 1991. Investigations of Proliferative and Senescent Callus of Soybean. **Physiologia Plantarum, Vol. 81 (4): 555-557.**

Khalil, S.M. K.T. Cheah, E.A. Perez, D.A. Gaskill, J.S. Hu, 2002. Regeneration of banana (*Musa spp.* AAB cv. Dwarf Brazilian) via secondary somatic embryogenesis. **Plant Cell Reports, 20:1128–1134.**

Kosmiatin, M., I. Mariska, I. Roostika, dan E. Gati, 2006. Pembentukan Pisang Ambon Toleran terhadap Penyakit Layu *Fusarium* melalui Variasi Somaklonal**. Zuriat 17 (1) :16-24**

Laporan Akhir Riset Unggulan Strategis Nasional 2003. Pengembangan Buah-Buahan Unggulan Indonesia http://pkbt.ipb.ac.id/pages/exsum/2003-nenas.pdf).

Lestari, E.G., 2006. Review: In Vitro Selection and Somaclonal Variation for Biotic and Abiotic Stress Tolerance. **Biodiversitas, 7 (3): 297-301.**

Matsumoto, K., M.L. Barbosa, L.A.C. Souza, J. B. Teixeira, 1995. Race 1 *Fusarium* wilt tolerance on banana plants selected by fusaric acid. **Euphytica 84:67-71.**

Purwati, R. D., U. Setyo-Budi, Sudarsono, 2007. Penggunaan Asam Fusarat Dalam Seleksi *In vitro* Untuk Resistensi Abaka Terhadap *Fusarium* oxysporum F.Sp. Cubense, **Jurnal Littri Vol.13 (2) : 64 – 72**

Ramírez-Villalobos, M., and E. de García, 2008. Obtainment of embryogenic cell suspensions from scalps of the banana CIEN-BTA-03 (Musa sp., AAAA) and regeneration of the plants. **Electronic Journal of Biotechnology Vol.11(5), Special Issue.**

Sidha, M., P. Suprasanna, V. A. Bapat, U.G. Kulkarni, B.N. Shinde, 2007. Developing Somatic Embryogenic Culture System And Plant Regeneration In Banana. **FOUNDER'S DAY SPECIAL ISSUE: Issue No 285,October 2007.**

Srangsam, A. and Kanchanapoom, K, 2003. Thidiazuron induced plant regeneration in callus culture of triploid banana (Musa sp.) 'Gros Michel', AAA group. **Songklanakarin J. Sci. Technol., 2003, 25(6) : 689-696.**

Widodo, S., C Sumardiyono, B. Hadisutrisno, 2003. Pengimbasan Ketahanan Pisang Terhadap Penyakit Layu *Fusarium* dengan *Burkholderia Cepacia*. **Agrosains 5 (2): 72-79.**

Wirakarnain, S., A.B.M.S. Hossain, S. Chandran, 2008. Plantlet Production through Development of Competent Multiple Meristem Cultures from Male Inflorescence of Banana, Musa acuminta cv. 'Pisang Mas' (AA). **American Journal of Biochemistry and Biotechnology 4 (4): 325-328.**

Zarmiyeni, Kasli, M. Kasim, I. Suliansyah, N. Nasir, Ishak, 2007. Seleksi In Vitro Untuk Resistensi Mutan Pisang Barangan Terhadap F. Oxysporum F.Sp. Cubense Menggunakan Asam Fusarat Sebagai Agens Penyeleksi. **Jurnal Ilmu-Ilmu Pertanian Indonesia. Edisi Khusus, No. 3, Hal. 427 – 431.**