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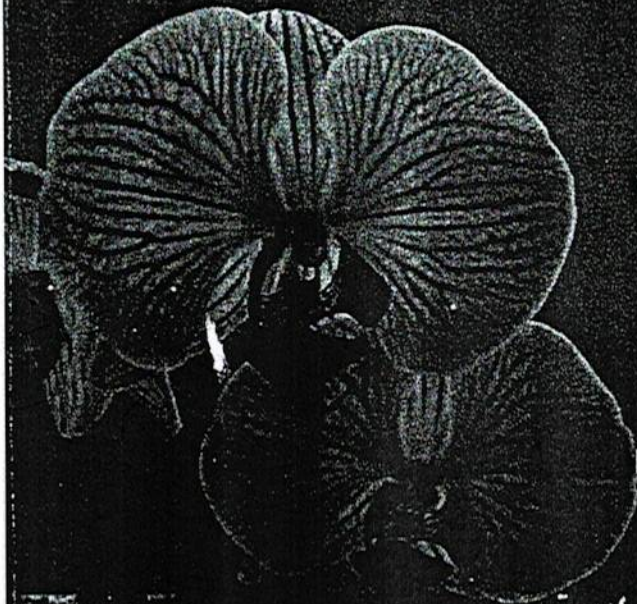
PROCEEDINGS

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EMBRYOGENIC CALLUS INDUCTION FROM MALE INFLORESCENCE OF LOCAL BANANA CULTIVARS WITH A VIEW TO PRODUCE *FUSARIUM* WILT RESISTANT PLANT *VIA IN VITRO* SELECTION²

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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 μ M). All treatment combinations were replicated 3 times. The nutrient medium used was Murashige and Skoog (MS-1962) supplemented with 7.5 μ M 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; Dimiyati, 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 (Dimiyati, 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 (Companiononi *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 chlamidospore which is 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 (Companiononi *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 through 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-acetic acid (IAA), 6-Benzylaminopurine (BAP), casein hydrolyzate, sucrose, agar, $HgCl_2$ 0.2%, sterilized distilled 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).

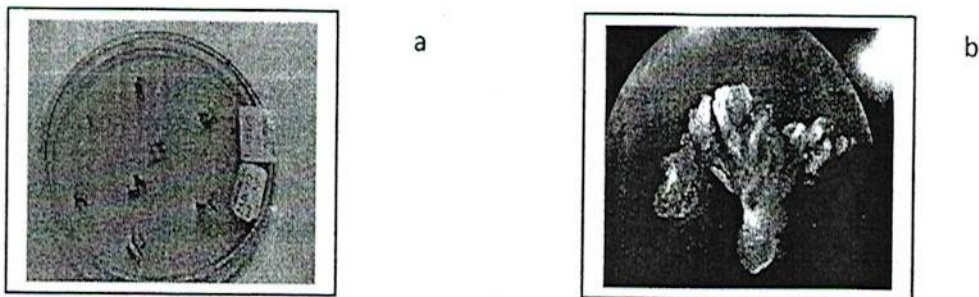


Figure 1. Callus formation: a) explants; b) callus formed on the explants surface

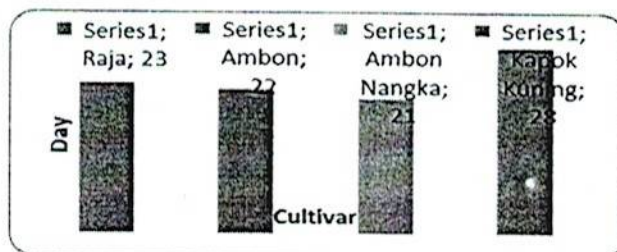


Figure 2. Callus formation time

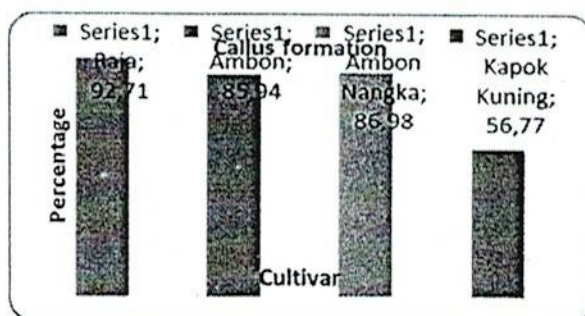


Figure 3. The percentage of explants forming callus

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.

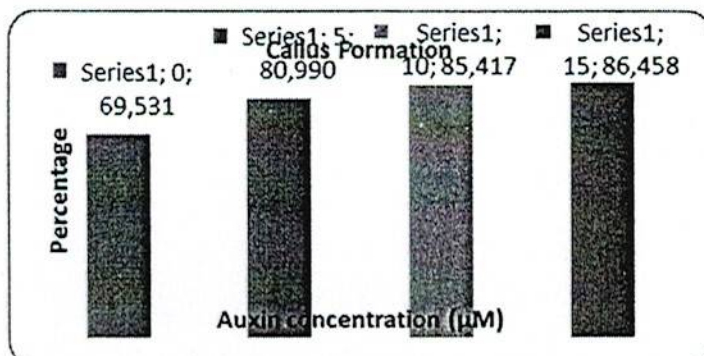


Figure 4. The effect of auxin concentration on the percentage of explants forming callus

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.

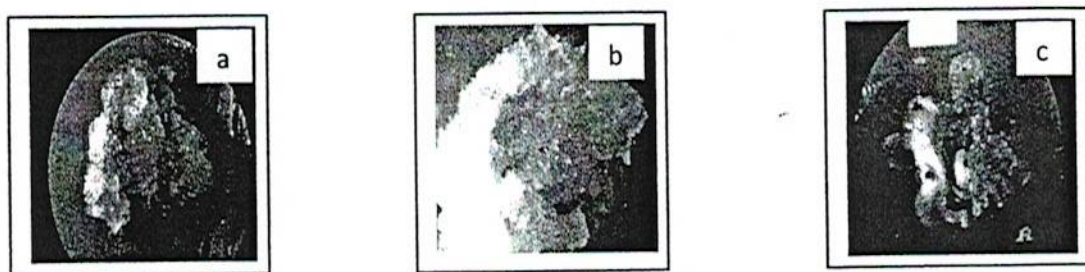


Figure 5. Banana callus type: a) embryogenic; b) proliferative; c) senescence

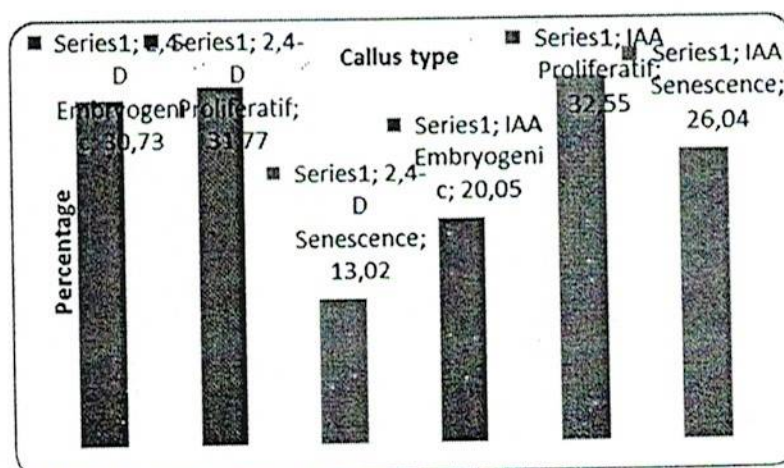


Figure 6. The effect of auxin concentration on the type of callus formed

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 μ M 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.

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