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Development of Malaysian Wild Bananas Seed progenies, *Musa acuminata* ssp. *malaccensis* and *Musa gracilis*.

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Embryo culture, micropropagation, wild bananas, Musa acuminata ssp. malaccensis, Musa gracilis.

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Abstract

An embryo culture and micropropagation technique was done to develop the clonal population of wild bananas, Musa acuminata ssp. malaccensis and Musa gracilis for breeding purpose. The aim of this study was to compare in vitro germination and multiplication rate of two Malaysian wild bananas species. Embryos were cultured at different days; starting from day one until day 30 of fruit's harvested. Result showed that the highest germination rate for both wild bananas was achieved from ripen fruit compared to unripe and over-ripen fruit. Germinated seedling were transferred onto MS media supplemented with 2 mg/L 6-benzylaminopurine (BAP) for several times to generate explants with multiple shoots. M. gracilis showed high proliferation rate compared to M. acuminata ssp. malaccensis after first sub cultured. In subsequent culture, number of shoots was increasing in M. acuminata ssp. malaccensis but not in M. gracilis.

1. Introduction

Musa species commonly known as banana, is an important staple food crop botanically belongs to the Musaceae family in the order Zingiberales [1]. It was cultivated in more than 130 countries throughout tropical and subtropical countries [2]. The United Nations Food and Agriculture Organization (FAO) ranks bananas as the world's eight most important food crops and the fourth most important agricultural crop in the world's least-developed countries [3].

Malaysia is one of the important centers of origin and diversity for both wild and cultivated bananas [4]. There are three wild *Musa* species indigenous of Malaysia, *Musa acuminata*, which is the ancestor of cultivated bananas, and an ornamental *Musa gracilis* and *Musa violascens* [5]. In Malaysia, banana is mainly cultivated on smallholdings for local consumption and export markets [2]. However, most cultivated bananas in Malaysia are susceptible to a range of serious and unbearable diseases. The most serious banana disease is known as *Fusarium* Wilt or Panama Disease which caused by the soil-borne pathogen, *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FOC TR4) [6].

Since the appearence of this disease, various control strategies like crop rotation [7], soil fumigation [8], flood fallowing [9], and the use of organic amendments [10] were tried and they have not been efficient and only provide temporary solution in managing *Fusarium* Wilt. The best approach is planting resistant varieties to replace varieties that susceptible to this pathogen. Previous studies showed natural disease resistance was present in wild bananas [11]. The three Malaysian wild bananas are offering a possible source of resistance to the disease, and would potentially be a good plant material for breeding and genetic strategies as they are fertile and produce a large number of seeds [12]. As genetic improvements of banana using conventional breeding are very difficult due to low fertility, polyploidy, long generation time and large area requirement for field-testing [13]. Application of biotechnology approaches such as genetic transformation, molecular markers micropropagation and embryo culture are very crucial to create new elite varieties that resistant to diseases [14].

Banana micropropagation have been widely studied as an alternate method for mass-scale production of high quality plant material [15] and this

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application has greatly improved *Musa* germplasm handling and played a key role in banana improvement programs worldwide [14][16]. The success of embryo culture and regeneration of plantlet depends on the maturity of seed and composition of culture medium [17].

The objectives of the present study were to determine the best fruit ripening stage after harvested to obtain seeds of Malaysian wild bananas, *M. acuminata* ssp. *malaccensis* and *M. gracilis* with high viability to compare *in vitro* germination rate of wild bananas and to investigate the influence of reducing apical meristem by subculturing to induce multiple shoots.

2. Materials and Methods

2.1. Plant materials

Mature seed samples of diploid wild banana were used. Samples for *M. gracilis* and *M. acuminata* ssp. *malaccensis* were harvested from Lojing Highland, Kelantan and infected area of *Fusarium* Wilt at Jelawang, Gua Musang, Kelantan, respectively. After harvested, fruits were stored at room temperature, 30-35°C.

2.2. Extraction of embryos and formation of shoots

The clonal seed progenies were developed through embryo culture and micropropagation [18]. Fruits collected were thoroughly washed to extract seed from the fruit skin and pulp. Under aseptic condition, seeds were soaked in 1.4% sodium hypochloride for 10 minutes and quick rinsing with 70% ethanol. The seed coat was broken apart to expose the embryo. The embryo was removed from the seed coat before transferring it onto fresh basal Murashige and Skoog (MS) media and kept in the dark for stimulate germination of embryos. After formation of shoot, culture were kept under 16 hours of light. Embryos were cultured at different days for thirty days; start from the day 1 until day 30 of fruit harvested and stored.

2.3. Multiplication of plantlets

Leaves and roots of germinated explants were trimmed before transferred onto fresh MS media supplemented with 2 mg/L 6-benzylaminopurine (BAP) to reduce apical meristem for induction of multiple shoots. Explants were subcultured several times to generate the clonal populations.

3. Results and Discussion

3.1 Embryo culture

Mature seeds were used for embryo culture of wild bananas because germination of immature embryos produce weak and often unviable plants [17]. Two population of *M. gracilis* and *M. acuminata* ssp. *malaccensis* were developed based on days of storage, at room temperature, 30-35°C.

The germination rate for both wild bananas were assessed after four weeks of embryo culture on basal MS media. The embryos went through different transition before the final seedlings growth as shown in Figure 1. The first changes observed were the yellowing appearance and swelling of embryos as describe by [18] [19] . Then, shoot primordial start to appeared and followed by emergence of roots primordial. After 2-3 weeks, plant-like structure appeared consisting of a prominent shoots, which bears an adventitious root system at it base. In a month, the roots system was replaced by thick, long and less branching roots which become the basis for the formation of a mature root system.

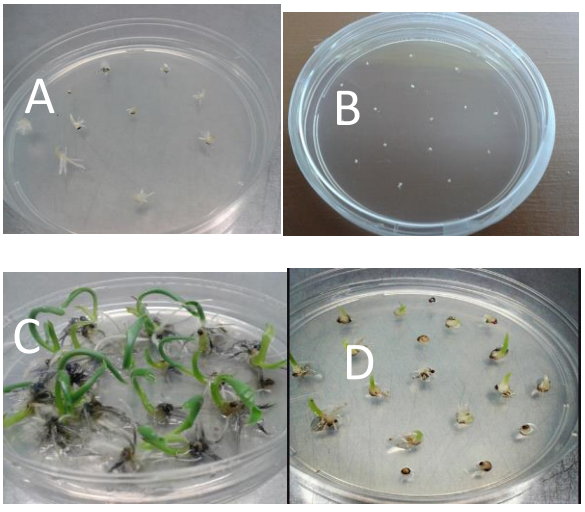

Figure 1: Different transitions of embryos were generated into plantlets. A) Embryos were cultured on basal MS media. B) Roots and shoots primordial started to appear C) prominent shoots and adventitious roots system D) plantlet ready to subculture.

There were differences of the germination rate for both species. Based on Table 1, the germination rate of M. gracilis was higher compared to M. acuminata ssp. malaccensis. According to [20], the germination rate of these wild bananas differs between harvest lots,

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which depends on fruit maturity at the time of harvest, post-harvest, physiological age of the seed and storage.

Table 1: Total number of embryos germinated and germination rate of M. gracilis and M. acuminata spp. malaccensis after four weeks of cultured.

Populations	Total embryos cultured	No. of germinated embryos	Germination Rate (%)
Musa gracilis	600	487	81.2
Musa acuminata spp. *malaccensis*	600	356	59.4

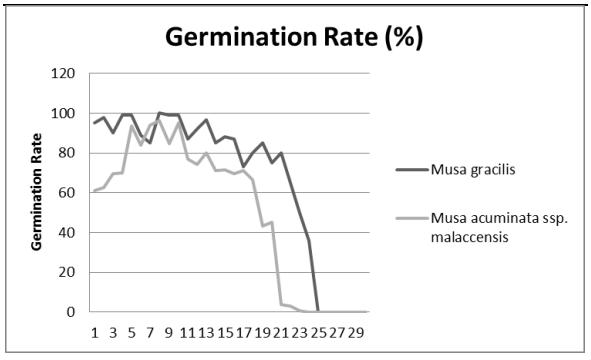

Figure 2 showed the germination rate of both species for embryos cultured from the day 1 until day 30 of fruits harvested and stored. From this results, the effect of ripening stages and storage can be determine. From the result, for M. gracilis, the highest germination rate was achieved at day 8 (100%) whereas M. acuminata ssp. malaccensis at day 9 (96%). Optimum germination rate of embryos cultured were obtained from day 5 until day 21 after harvesting. As mature fruits were harvested before the ripening process has begun, the process of fruits naturally ripen during the storage [21] (Figure 3). In observation, high germination rate of embryos cultured were obtained from ripen fruit. From previous study by [22], seed development were complete at fruit ripening not at maturity stage. However, both wild bananas showed drastically decreasing of germination rate at the end of month.

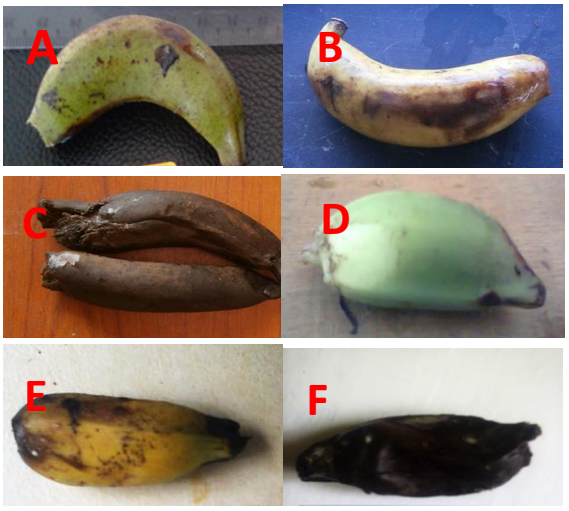

Figure 3 In vitro growth germination rate of two wild bananas, M. acuminata ssp. malaccensis and M. gracilis

3.2. Multiplication

There are several reasons that lead to this situation. The first important reason was embryos were cultured from over-ripen fruits, which the moisture content of the fruits already lost [23]. Hence, seeds were dried and viability of embryos to germinate were also decreased. Moreover, this low embryos germination were happened because of contamination rate were very high due to insect damage that caused by insect larvae, in particular from fruit fly during improper storage. Development of clonal seed progenies of wild bananas can be achieved by production and proliferation of axillary shoots from single individual. Shoot of germinated explant were trimmed and transferred onto fresh MS media supplemented with 2 mg/L BAP to induce multiple shoots. Data for each subcultured were obtained after 4 weeks of explants were transferred onto new fresh media. Table 2 showed number of explants with multiple shoots and number of shoots produce per explants for two wild bananas. . From the results, higher proliferation rate were obtained from explants of *M. gracilis* compared to *M. acuminata ssp. malaccensis* (Figure 4).

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Table 2: Numbers of explants with multiple shoots and average of shoots per explants of two wild bananas, M. acuminata ssp. malaccensis and M. gracilis.

	Musa gracilis		*Musa acuminata ssp. malaccensis*	
	No of explant with multiple shoots	Average no. of shoots per explant	No of explant with multiple shoots	Average no. of shoots per explant
First Subculture	65	4	7	2
Second Subculture	74	4	15	5
Third Subculture	95	5	36	10

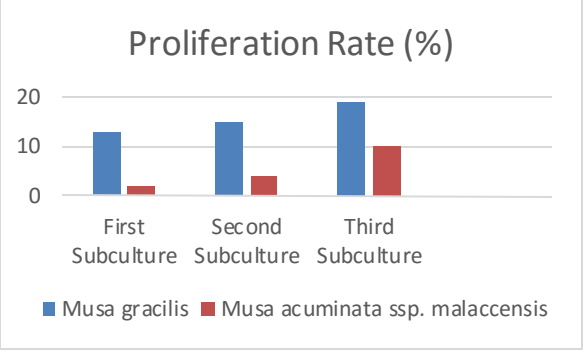

Figure 5: Proliferation rate of two different population of wild bananas, *M. gracilis* and *M. acuminata* ssp. *malaccensis* after first, second and third subculture.

Plant growth regulators (PGR), cytokinins such as 6-benzylaminopurine (BAP) were known to reduce apical meristem dominance and induce axillary shoots formation from meristematic explants in banana (24). Moreover, BAP is most widely used due to low cost and high effectiveness. From previous study, most of the species response well to BAP and with sufficient concentration of BAP were used, plantlets produced many axillary shoots and elongate [25]. From the results obtained, explants of *M. acuminata* ssp. *malaccensis* has less number of multiple shoots compared to *M. gracilis* because the apical meristem were very dominant. Shoots were elongate but not produce axillary shoots. Hence, it can be conclude that concentration of 2 mg/L BAP was optimal to *M. gracilis* but not to *M. acuminata* ssp. *malaccensis*.

Conclusion

Embryo culture and micropropagation are very useful in production of clonal seed progenies of wild bananas. The results of this study indicate that fruit ripening stages and storage of fruits harvested can influence germination rate of embryos for both species. Embryos were cultured from seeds of ripen fruits gave higher germination rate compared to unripe and over-ripen fruits. *M. gracilis* produced higher proliferation rate compared to *M. acuminata* ssp. *malaccensis*, which offering a potential source for next banana breeding. Study on resistance towards FOC TR4 especially for *M. gracilis* need to be considered as further research to overcome

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