Effects of Genotype and Intercropping with Chinese Chives (*Allium tuberosum*) on Fusarium wilt Tropical Race 4 in Banana

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Abstract

Fusarium wilt is one of the major threats faced by the banana industry in Malaysia. The causal pathogen is a soil-borne fungus, *Fusarium oxysporum* f. sp. *cubense* which is able to remain active in the soil for a long time. An integrated strategy is needed to battle this disease. A combined approach of screening local banana cultivars and intercropping with Chinese chives (*Allium tuberosum*) was tested. Three genotypes, i.e. 'Grande Naine' (AAA genome, Cavendish subgroup), 'Berangan Intan' (AAA genome) and 'Lemak Manis' (AA genome) were evaluated in a Fusarium hot-spot area. The 'Lemak Manis' cultivar survived with two cycles of ratoon harvested, whereas somaclonal variant of 'Berangan Intan' was successfully screened and selected for second round of microprogation via tissue culture technique. Intercropping with Chinese chives was not found to contribute to controlling the disease when intercropped with 'Berangan Intan' and 'Lemak Manis' but was able to suppress the disease when intercropped with 'Grande Naine'.

INTRODUCTION

Globally, banana (*Musa* spp.) is ranked within the top ten most important food crops in the world with the top three being rice (*Oryza sativa*), wheat (*Triticum* spp.) and soybean (*Glycine max*). India and China are the highest banana producing and consuming countries in the world (FAOSTAT, 2012). In Malaysia, banana is the second most widely cultivated fruit with a total production of 535, 000 metric tonnes on about 10% (33,584 ha) of the total fruit area (297,860 ha) (Mohamad Roff et al., 2012).

However, the banana industry faces major threats in its cultivation, such as pests, diseases and lack of fertile soils. One of the major threats faced by the banana industry in Malaysia is the systemic Fusarium wilt (also called Panama disease). The causal pathogen is a soil-borne fungus, *Fusarium oxysporum* f. sp. *cubense* (Foc) with genetic variability as there are four different races (Hermanto et al., 2012) and is able to remain active in the soil for a long time (Sreeramanan et al., 2006). One of the races is the Tropical Race 4 (TR4) that has struck all commercial banana cultivars.

There is no chemical control available for Fusarium wilt, and our best option to manage the disease is to plant cultivars that are resistant to the pathogen (Javed et al., 2004). However, the suitability and potential as commercial clones of these resistant cultivars is often lacking. Therefore, the selection of somaclonal variants resistant to Foc TR4 in popular local cultivars provides a strategy to obtain resistant cultivars with commercially acceptable qualities. Three genotypes, i.e. 'Grande Naine' (AAA genome, Cavendish subgroup), 'Berangan Intan' (AAA genome) and 'Lemak Manis' (AA genome) were evaluated in a Fusarium hot-spot area for this report.

Studies using essential oil and crude extract of several *Allium* species have demonstrated their antibacterial, antifungal, antioxidant and cytotoxic activities (Najjaa et al., 2007). Research conducted by the Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences (GAAS) showed that banana crop rotation systems planted with Chinese leek or Chinese chives (*Allium tuberosum*) in soil infected with Foc was able to effectively suppress the development of Fusarium wilt (Huang et al., 2012). Wibowo (2012) repeated the trial in Indonesia using four different species, namely *A. tuberosum*, *A. fistulosum*, *A. cepa* and *A. wakegi*. The results showed that up to 12 months after planting, these 4 species of *Allium* intercropped with banana cultivar 'Ambon Kuning' (AAA genome), only *A. tuberosum* and *A. cepa* were able to suppress the incidence of banana Fusarium wilt disease (by 46% and 33%, respectively), when compared to the control treatment. It was suspected that the bulb extracts of *Allium* spp. have different abilities to suppress the mycelial growth and spore germination of Foc Race 4. According to Zhang (2013), the reduced incidence of Fusarium wilt in the presence of Chinese chives could be due to the sulfur volatiles causing inhibitory effects on Foc mycelial growth and spore germination.

Here, we combined the approach of screening three local banana cultivars and intercropping with Chinese chives.

MATERIALS AND METHODS

The trial was carried out in Jendarata Estate, Teluk Intan, Perak, Malaysia (19.40 m above sea level, 2,400 mm annual average rainfall, average min/max temperature 20.4°C and 32.5°C). The soil is classified as 'Briah' Series, marine clay mineral soil with a pH of 5.0.

Trial Design

Three cultivars were used in this trial, namely 'Grande Naine', 'Berangan Intan' and the control 'Lemak Manis' as it is highly resistant to Fusarium wilt.

Suckers were brought in for shoot-tip meristem culture and sub cultured for six months in semi-solid media. The plantlets with hairy roots were sent for acclimatization to the nursery. The plantlets were kept for hardening in the nursery for 64 days and then used as planting materials in the field. Half of the plants were inoculated with Foc at planting (see below). The tissue cultured planting materials were planted in the field in a split-plot design in July 2012, with three blocks and 12 replications (plants) per cultivar per block (36 plants per cultivar in total). A border of 'Pisang Tanduk' (ABB 'Horn') was planted. Plant spacing was 2.4 m within a row and 1.5 m between rows.

At planting, 50 g each of rock phosphate and ground magnesium lime were applied in the planting hole. Fertilizer application and all management practices followed the protocol established in United Plantations Berhad, with no application of fungicides in the soil or on the foliage.

Inoculation with *Fusarium oxysporum* f. sp. *cubense*

Inoculum was increased by adding chopped, infected banana rhizomes and pseudostems from neighboring plants in the same field into the planting hole prior to planting. Each plant in the experimental plot received 1000 g of this inoculum. The plants were manually watered for the first two months and thereafter, the field drains were irrigated with river water (Jendarata River, Teluk Intan).

Intercropping with *Allium tuberosum*

The seeds were procured from Soon Huat Seeds Co. Sdn Bhd in Lebuh Pantai, Penang Island. The seeds which originated from China were propagated in the nursery in Jendarata Estate. The seeds were soaked in water for two hours and sown in perforated plastic trays filled with coarse riverine soil. The seeds germinated after two months and at four months, the seedlings were transplanted to the field. The seedlings were planted at four points around the banana plants.

Evaluation of Fusarium Wilt

During the growth period, the survival rate of the three cultivars was recorded. Three external symptoms of Fusarium wilt were recorded: yellowing of foliage, splitting of pseudostem and collapse of petiole with green lamina. These were recorded until the plants withered and fell or until harvest, whichever came first.

With a digging bar, the rhizome base was dug up and removed from soil. Transverse sections of the rhizome are cut and the upper section of the cut section was examined. The extent of vascular discoloration is noted on the scale of 1 to 5 (Orjeda and Promusa, 1998). For this report, to verify the presence of the disease, only the internal ratings of disease severity of the scale 5 were recorded at harvest or, if the plants were infected and fell before yielding.

Agronomic traits of number of functional leaves, height and girth of pseudostem at harvest, bunch weight, number of hands and weight of the third hand were recorded. The number of fingers on the third hand, length and width of the fingers on the third hand were also determined after the bunch was harvested.

PCR Analysis

Isolates of Foc were recovered from the pseudostem of infected and fallen banana trees. PCR was run using different specific primers described by Dita et al. (2010).

RESULTS

PCR was performed using DNA from infected rhizomes. As shown in Fig. 1a, the PCR results were positive for tropical race 4. The bands were 463 bp similarly obtained by Dita et al. (2010).

All plants of all cultivars survived at the first month after planting (Fig. 1b). At 101 days after planting, the overall survival rate across all cultivars was comparable between the *A. tuberosum* treatment and the control treatment at (46.3% and 48.1%, respectively). ‘Lemak Manis’ had the highest survival rate in both treatments (at 75% and 83% in the *A. tuberosum* and control treatment, respectively). ‘Berangan Intan’ had the lowest survival rate in both the treatments (25% and 22% in the *A. tuberosum* and control treatment, respectively). There was no difference in the survival rate between the two treatments for ‘Grande Naine’ at 39% for both treatments.

At 165 days after planting, the overall survival rate across all cultivars was not significantly different between the *A. tuberosum* treatment and the control treatment (at 29.6% and 31.5%, respectively). ‘Lemak Manis’ had the highest survival rate in both treatments (66.7% and 72.2% in the *A. tuberosum* treatment and control treatment, respectively) and the difference between the treatments was not significant. ‘Berangan Intan’ had the lowest survival rate in both treatments (0% and 8.3% in the *A. tuberosum* treatment and control treatment, respectively) with a significant difference between the treatments. ‘Grande Naine’ had a higher survival rate in the *A. tuberosum* treatment than

in the control treatment (22.2% and 13.9%, respectively) with a significant difference between the treatments. *A. tuberosum* treatment did not suppress the incidence of Fusarium wilt in 'Lemak Manis' or 'Berangan Intan'. The survival rate of 'Grande Naine' in the *A. tuberosum* treatment at 22.2% was significantly higher than in the control treatment (13.9%).

At six months after planting, though 'Lemak Manis' had the lowest number of dead plants in both treatments, the surviving plants of this cultivar had the highest expression of external symptoms, like yellowing of foliage and petiole collapse in both treatments (Fig.1c). Exactly the opposite was observed for 'Berangan Intan'. On this cultivar, there were no signs of splitting of pseudostem for the remaining plants in the control treatment. 'Grande Naine' expressed similar expression of external symptoms such as yellowing of foliage and petiole collapse, in both treatments.

Disease severity of stage 5 in 'Berangan Intan' was the highest as this cultivar had the highest number of dead plants (Table 1). 'Lemak Manis' had the lowest internal symptoms, at only 2.8%, which was observed in the fallen plants and none was observed in the non-fallen plants upon harvesting. 'Grande Naine' exhibited more severe vascular discoloration in the fallen plants than the non-fallen plants.

There were no significant differences in the average pseudostem height and girth at harvest between the *A. tuberosum* treatment and the control for 'Lemak Manis' (Table 2). However, the average bunch weight and the average third hand weight were significantly better in the control treatment than in the *A. tuberosum* treatment (6.0 kg compared with 4.5 kg, and 0.6 kg compared with 0.5 kg, respectively). There were also no significant differences in the pseudostem height and girth at harvesting for 'Grande Naine' between *A. tuberosum* treatment and the control. The average bunch weight and the mean third hand weight were better in the control treatment (18.6 kg and 1.6 kg, respectively) than in the *A. tuberosum* treatment (11.2 kg and 1.5 kg, respectively). As all the 'Berangan Intan' in the *A. tuberosum* treatment did not come into bearing, only the 'Berangan Intan' bunches in the control treatment were evaluated at harvest. The average pseudostem height and girth at harvest for 'Berangan Intan' was the highest amongst all three cultivars evaluated. The average bunch weight and mean third hand weight of the surviving 'Berangan Intan' was low at 6.8 kg and 1.0 kg as compared to the average in a non Fusarium hot spot area. Generally, the mean bunch weight of 'Berangan Intan' in a non-Fusarium hot spot area is 18Kg and average third hand weight of 2Kg.

DISCUSSION

In United Plantations Berhad, 'Berangan' was identified to have the greatest potential for commercial cultivation based on the high bunch weight and better storage and eating qualities compared to the other local cultivars. The elite 'Berangan' cultivar selected with more than 18 Kg in bunch weight and more than 7 hands per bunch were designated as 'Berangan Intan' (Ho and Singh, 2000). However, this cultivar is also most susceptible to Fusarium wilt.

With this study, we have shown that the degree of Fusarium wilt infection is highly cultivar dependent. It was observed that all 'Berangan Intan' succumbed to the disease in this field trial after only six months in the field which was also reported by Jamaluddin et al. (2001) and Liew et al. (2000). The high percentage of fallen 'Berangan Intan' exhibiting internal symptoms at Stage 5 (87.5%) indicates the severity with which the disease attacks this cultivar. However, three plants survived and came into fruiting. The suckers of these plants have been brought in for tissue culture and will be evaluated

in the same Foc hot-spot area. Although improvement of banana by production of novel variants via somaclonal variation has been well documented, stability of the genetic changes is an important factor in crop improvement programs (Martin et al., 2006). Hence, a second round of 'Berangan Intan' microprogation via tissue culture technique is underway and will be field tested in the same Foc hot-spot area.

As a group, the edible diploid 'Lemak Manis' cultivar are less hardy and bear fewer and smaller fruits than triploids (Stover and Simmonds, 1987). This cultivar is the least robust among all three cultivars evaluated in the field trial. However, as the suckers of the 'Lemak Manis' were previously taken from surviving trees through selection pressure at the same Fusarium wilt hot-spot area, this cultivar repeatedly showed resistance to the disease (Liew et al., 2000). Plants that are selected through selection pressure of somaclonal variation for Fusarium wilt tolerance is known to be able to survive and fruit (Hwang, 2004). At the point of reporting, the surviving plants of 'Lemak Manis' cultivar is yielding from the second ratoon. The weak agronomic trait found in 'Lemak Manis' cultivar is typical of a diploid banana.

The 'Grande Naine' is a triploid variant (AAA genotype) in the Cavendish subgroup, medium-dwarf varieties. These varieties have more commercial importance because of the low height with no sacrifice in finger length (Stover and Simmonds, 1987). In this field trial, there was a distinct difference at six months when survival rate of plants treated with *A. tuberosum* was eight percent higher than the control. It was also noted that in the 'Grande Naine' cultivar, half of all the plants exhibited more external infection symptoms, such as the yellowing of the foliage and petiole collapse, in the control treatment than the *Allium tuberosum* treatment, thus deducing certain degree of control when intercropped with Chinese chives.

Vegetative measurement of all cultivars between treatments did not show any significant difference but the bunch weight was always significantly better in the control treatment than in *A. tuberosum* treatment.

Further study is underway to intercrop *A. tuberosum* at the hardening stage before field planting to better control the disease. Methodological challenges create barriers in the discovery of disease and control mechanisms that involve plant volatiles due to the extreme difficulties in controlling soil-borne fungi because of their extensive mycelia and numerous spores above and underground (Hu et al., 2006). It is hypothesized that the intercropped banana plants will be in a buffer zone when planted in the hot-spot area and would be able to better suppress the incidence of Fusarium wilt. Studies of allicin concentration and sulfur volatiles of the Chinese chives are also being conducted to understand their role(s) in controlling this virulent pathogen.

CONCLUSIONS

Surviving 'Berangan Intan' have been selected through selection pressure for Fusarium wilt disease tolerance for second round of micropropagation via tissue culture technique and will be field tested at the same Foc hot spot area. *A. tuberosum* was intercropped with 'Grande Naine' cultivar and was able to suppress the incidence of Fusarium wilt. Intercropping with *A. tuberosum*, however proved futile in controlling Fusarium wilt for 'Berangan Intan'. Intercropped 'Berangan Intan' cultivar with *A. tuberosum* at the hardening stage prior to field planting is ongoing as this cultivar has the most potential in commercial cultivation in Malaysia.

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Literature Cited

Dita, M.A., Waalwijk, C., Buddenhagen, I.W., Souza Jr., M.T., Kema, G.H.J. 2010. A Molecular diagnostic for Tropical Race 4 of the banana Fusarium wilt pathogen. Plant Pathol. 59:348-357.

FAOSTAT. 2012. Food and Agricultural Organization Statistics. http://faostat.fao.org/site/291/default.aspx

Hermanto, C., Djatnika, E.I., Emilda, D., Mujiman, and Subhana. 2012. Pre-planting treatments for management of banana Fusarium wilt. ARPN J. of Agr. .Biol. Sci. 7(4): 260-265.

Ho, Y.W. and Singh, G. 2000. United Plantations Berhad's Contribution to the Banana Industry. Proceedings of the first National Banana Seminar at Awana Genting and Country Resort. UPM, Serdang.

Hu, G., Lu, Y. and Wei, D. 2006. Chemical characterization of Chinese chive seed (*Allium tuberosum* Rottl.). Food Chem.99:693-697.

Huang, Y.H., Wang, R.C., Li, C.H., Zuo, C.W., Wei, Y.R., Zhang, L. and Yi, G.J. 2012. Control of Fusarium wilt in banana with Chinese leek. Eur. J. Plant Pathol. 134(1):87-95.

Hwang and Ko (2004). Cavendish banana cultivars resistant to Fusarium wilt acquired through somaclonal variation in Taiwan. *Plant Diseases*. 88:580-588.

Jamaluddin, S.H., Masdek, N.H. and Ibrahim O. 2001. Preliminary survey of banana Fusarium wilt disease in Malaysia – Implications on production and the industry. Banana Fusarium wilt Management: Towards Sustainable Cultivation INIBAP-ASPNET, Los Baños.

Javed, M.A., Chai, M. and Othman, R.Y. 2004. Study of resistance of *Musa acuminata* to *Fusarium oxysporum* using RAPD markers. *Biologia Plantarum* 48(4):93-99.

Liew, K.W., Chai, M. and Ho, Y.W. 2000. Status of banana Fusarium within Malaysia. Proceedings of the first National Banana Seminar at Awana Genting and Country Resort. UPM, Serdang.

Martin K.P., Suneesh Kumar P., Zhang, C.L., Slater, A. and Madassery, J. 2006. RAPD analysis of a variant of Banana (*Musa* sp.) cv. Grande Naine and its propagation via shoot tip culture. In vitro Cell. Dev. Biol - Plant 42:188-192.

Mohamad Roff, M.N., Tengku Abdul Malik, T.M. and Sharif H. 2012. Challenges to banana production in Malaysia: A threat to food security. The Planter 88(1030):13-21.

Najjaa, H., Neffati, M., Zouari, S. and Ammar, E. 2007. Essential oil composition and antibacterial activity of different extracts of *Allium roseum L.*, a North African endemic species. Compt. Rend. Chim. 10:820-826.

Orjeda, G. 1998. Evaluation of *Musa* Germplasm for Resistance to Sigatoka Diseases and Fusarium wilt. INIBAP Technical Guidelines 3. IPGRI, Rome; INIBAP, Montpellier; CTA, Wageningen.

Sreeramanan, S., Maziah, M., Sariah, M., Puad, M.P. and Xavier R. 2006. Bioassay method for testing Fusarium wilt disease tolerance in transgenic banana. Sci. Hort. – Amsterdam 108:378-389.

Stover, R.H. and Simmonds, N.W. 1987. Bananas. Thrid Edition. John Wiley and Sons, New York.

Wibowo, A., Alboneh, A.R., Subandiyah, S., Pattison, A. and Molina A.B. 2012. Increased soil suppressiveness to banana Fusarium wilt disease through banana intercropping with *Allium* spp. Presentation at International Banana Symposium: Banana Improvement, Health Management, Use Diversification and Adaptation to Climate Change. Taiwan, 19-22 November 2012.

Zhang, H., Mallik, A. and Zeng, R.S. 2013. Control of Panama disease of banana by rotating and intercropping with Chinese chive (*Allium tuberosum* Rottler): Role of plant volatiles. J. Chem. Ecol. 39:243-252.

Tables

Table 1. Evaluation of stage 5 internal symptoms in plants of three banana cultivars, at time of harvest or, if they had fallen before, at the time of falling.

Cultivars	**Number of Plants evaluated**	**Percentage of plants with stage 5 internal symptoms (%)**	
		Fallen	**Non-fallen**
'Lemak Manis'	72	2.8	0
'Grande Naine'	72	27.8	13.9
'Berangan Intan'	72	87.5	4.2

Table 2. Agronomic traits of three banana cultivars during harvest.

Treatments	**'Lemak Manis' Control**	**'Lemak Manis' *Allium tuberosum***	**'Grande Naine' Control**	**'Grande Naine' *Allium tuberosum***	**'Berangan Intan' Control**	**'Berangan Intan' *Allium tuberosum***
FLH	7	8	8	7	7	
PHH (m)	1.7	1.8	2.1	2.0	2.2	
PGH (m)	0.43	0.45	0.56	0.55	0.68	
BW (kg)	6.0	4.5	18.6	11.2	6.8	
C	7	7	8	8	6	NA
TCW (kg)	0.6	0.5	1.6	1.5	1.0	
F	15.7	16.1	14.0	14.0	15.0	
LF (cm)	7.4	7.5	15.5	14.5	9.7	
WF (cm)	2.6	2.1	2.7	2.1	2.3	

FLH – Functional leaves at Harvesting, PHH – Pseudostem height at Harvesting, PGH – Pseudostem girth at Harvesting, BW – Bunch Weight, C – No. Of Combs, TCW – Third comb Weight, F – No. of Fingers, LF – Length of Fingers, WF – Width of Fingers. NA – Not Available.

Figures

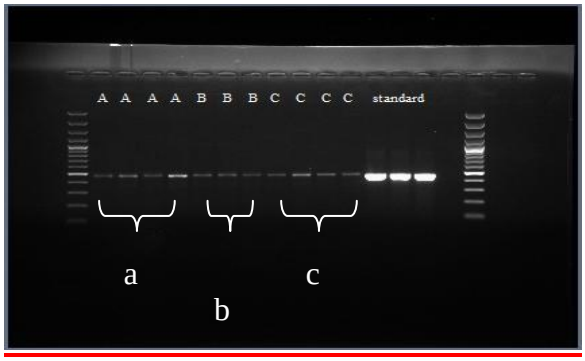

Fig. 1a. PCR product of *Fusarium oxysporum* f. sp. *cubense* from infected banana pseudostem showing bands at 463 bp.

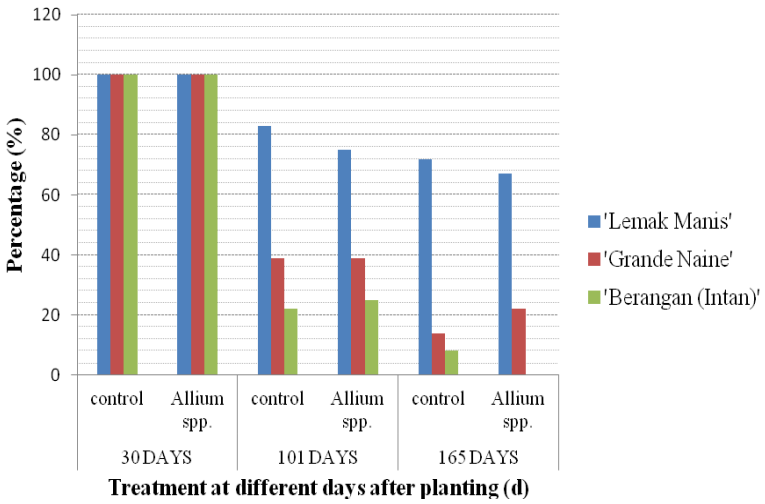

Fig. 1b. Survival rates of three banana cultivars, with and without intercropping with *Allium tuberosum* at 30, 101 and 165 days. n = 216, df = 5, chi^2 = 81.69, p<0.05.

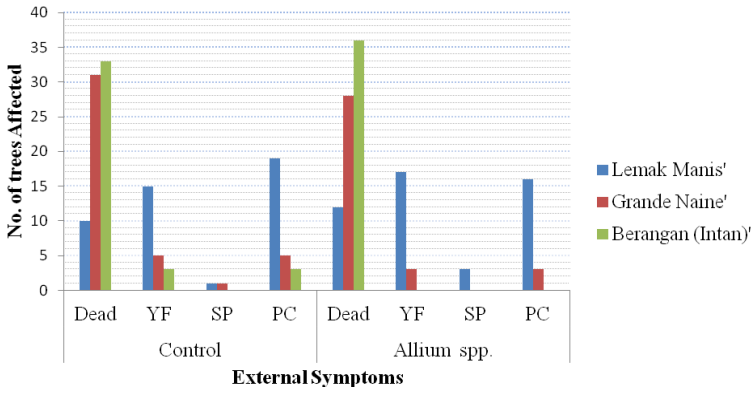

Fig. 1c. External symptoms of Fusarium wilt on three banana cultivars, with and without intercropping with *Allium tuberosum* treatment, at six months after planting. YF - Yellowing of Foliage, SP - Splitting of Pseudostem, PC - Petiole Collapse. n = 94, df = 10, chi^2 = 19.37, p<0.05.