Screening of banana clones for resistance to fusarium wilt in China

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Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) has been a serious problem in some localities in south China banana-producing regions since 1996 (Qi 2001). Race 1 of the fusarium wilt was reported earlier (Zeng *et al*. 1996), whereas race 4 (VCG 01213, 01216) was identified only recently. They are fast spreading and very difficult to control.

Although the plant age, weather, general conditions of root system, the physical state of soils, drainage, the nutrient status of the soil, and the amount of inoculum greatly impact the occurrence and course of the wilt disease, the clone grown is recognized the most important factor to decisively influence the course of infection (Stover and Simmonds 1987). In order to search for different sources of resistance, IMTP trials for fusarium wilt supported by INIBAP and Guangdong province were conducted between 2002 and 2004 in Panyu and Dongguan of Guangdong Province. Fifteen introduced clones plus one local cultivar were included in the evaluation in Panyu. Another 28 Cavendish cultivars were evaluated in Dongguan. This paper is a report of the results of this trial.

Materials and method

Testing sites

The trials were carried out in Panyu and Dongguan, Guangdong Province, which are located in the center of the Pearl River Delta of Guangdong Province. The field plot for this trial was rented from farmers, and has been seriously infested with Foc, with about 50% of banana trees being devastated in the previous crop.

In November 2000, two fusarium wilt samples collected in Panyu and Zhongshan of Guangdong Province were sent to Australia via Mr Bob Williams. On 14 December 2000, Drs Natalie Moore and Desley Tree identified two VCG groups: 01213 and 01216 ('tropical' race 4 strains), which were the same as those in Malaysia and Taiwan (Tang and Hwang 1999; Lee *et al.* 2001).

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Planting materials

Fifteen cultivars from INIBAP Transit Center (ITC) and one local variety, Baxijiao, were used in the Panyu trial. The ITC clones were 0505 (FHIA 02, AAAB), 0506 (FHIA03, AABB), 0570 (Williams, AAA), 0712 (AA cv Rose), 1122 (Gros Michel, AAA), 1123 (Yangambi KM5, AAA, Ibota), 1264 (FHIA-17, AAAA), 1265 (FHIA-23, AAAA), 1282 (GCTCV-119, AAA), 1283 (SH 3436-9, AAAA), 1297 (TMBx 5295-1, AAAB), 1307 (SH-3640), 1319 (FHIA-18), 1332 (FHIA-21, AAAB) and 1344 (CRBP 39, AAAB).

Five tubes each of banana clones were introduced from ITC on 4 September 2001. The buds in two tubes were multiplied in nine subcultures for sufficient plant numbers. After general quarantine procedures in pots and inside netted plastic houses, *in-itro* cultured plantlets were transplanted into plastic bags and used as planting materials after 60 days of hardening.

Trial design and tree management

Trees of 30-50 cm tall were planted in the field with a randomized complete block design on 17 August 2002. Six replication trees in each of three blocks were planted. Plant spacing was 2.0 m between trees, 1.5 m between the narrow rows and 2.0 m between wide rows. All management practices were applied uniformly over the whole trial site. Inoculum was increased by adding chopped, infected banana corms and stems from neighbouring orchards to the soil on 30 August 2002. Each plant in the experimental orchard received 1000 g of this inoculum. For other managements, the technical guidelines of Carlier *et al*. (2003) were generally followed, with no application of fungicides in soil or on foliage. Trees were irrigated with river water.

Data collection

Generally one investigation each month after December 2002 was carried out on fusarium wilt. One investigation on leaf spot diseases was done 6 months after planting. Due to its very light occurrence throughout the year and no visible difference among cultivars, the leaf spot disease is not analysed in this report. The means of data from each average of plots were listed and compared.

Investigation on fusarium wilt. During the growth period, three external symptoms were recorded on fusarium wilt: yellowing of erecting leaf, splitting of pseudostem, and collapse of petiole with leaf lamina in green colour. With a sharp spade, banana trees were examined internally to verify the presence of the disease and internal ratings of

disease severity at harvest, or if plants are going to die before yielding fruit (Carlier *et al.*, 2003). However, due to two successive strong typhoons on 23/24 August and 12/13 September 2003, most of the mother plants fell down and did not reach the stage of harvest.

Investigations on agronomic characteristics. Agronomic characteristics of standing leaf number, height of pseudostem at flowering time, bunch weight, number of hands and fingers, and when applicable, number of functional leaves at harvest were recorded.

Results and discussion

Most trees of 'AAcv Rose', 'Gros Michel' and 'Yangambi Km 5' grew slowly and weakly, indicating that they may not be accustomed to the environmental conditions in the experimental field.

External and internal symptoms of fusarium wilt disease in bananas

There were three types of symptoms in typical banana fusarium wilt disease in different genome groups. They are: upward leaf yellowing in Cavendish type (Figure 1A); leaf petiole collapse in Fenjiao (Pisang Awak) type (Figure 1B); stem cracking in young trees (Figure 1 C); and vascular discoloration in stem and corm (Figure 1D). Diseased corms rotted under most conditions.

First external symptom was observed in 210-350 days in planting cycle (Table 1). 'Gros Michel' and TMBx 5295-1 were the earliest to show yellow leaves 210 days after planting. Gros Michel was severely stunted, showed internal necrosis, and never reached flowering stage. More or less external symptoms may be seen in other clones (Table 2). 'Baxijiao' and 'Williams' had the highest ratio of diseased trees, being 72.2% and 44.4% respectively. Their internal discoloration index was 2.8 and 2.5 (Table 2). High ratio of leaf yellowing in FHIA-03, 'Yangambi Km 5,' GCTCV-119 and FHIA-18 were recorded, yet their vascular discoloration was not visible. 'AA cv Rose' did not show any external symptoms although minimum internal vascular discolouration was observed.

Due to typhoon damage, only a few trees were confirmed to have died of fusarium wilt disease. There were no trees of FHIA-03, 'Yamgambi Km 5' and GCTCV-119 dead from wilt, which might be confirmed by the discoloration index (Table 2). However, 20 % of 'Baxijiao,' 'Williams,' SH3436-9, FHIA18 and CRBP39 trees died during the planting cycle. Seventy two percent of 'Williams' trees were destroyed by Foc.

Table 1. Days of plant disease resulting from fusarial wilt in the planting cycle in the IMTP3 trial conducted in Panyu, Guangdong Province. Planting date: 17 August 2002.

Clone	Days of disease from planting
FHIA-02	295±91
FHIA-03	314±62
'Williams'	352±47
AA cv Rose	348
'Gros Michel'	210
'Yangambi km 5'	348
FHIA-17	333±60
FHIA-23	340±33
GCTCV-119	348
SH3436-9	348
TMBx5295-1	210
SH-3640	348±0
FHIA-18	255±120
FHIA-21	320±57
CRBP39	327±52
'Baxijiao'	348

Table 2. Occurrence of fusarium wilt determined by external and internal symptoms in the planting cycle in the IMTP III trial conducted in Panyu, Guangdong Province. Planting date: 17 August 2002.

Clone	Number of plants evaluated	Plants with external symptoms %	Internal discoloration index
FHIA-02	18	22.2	2.8±1.7
FHIA-03	18	38.9	1.0
'Williams'	18	72.2	2.5±2.1
cv. Rose	18	0	1.8±1.1
'Gros Michel'	18	100.0*	3.7
'Yangambi km 5'	12	33.4	1.0
FHIA-17	18	25.0	4.8
FHIA-23	18	38.9	1.5
GCTCV-119	18	27.8	1.1±0.2
SH3436-9	18	5.6	1.6±1.0
TMBx5295-1	18	38.9	2.0±1.4
SH 3640	18	11.1	2.7±2.1
FHIA-18	18	38.9	1.0±0.0
FHIA-21	18	27.8	2.6±1.4
CRBP39	24	16.7	2.8±1.1
'Baxijiao'	18	44.4	2.8±1.1

*Plants were severely stunted and did not reach flowering stage.

Figure 1. The external and internal symptoms in banana fusarium wilt disease. A. Petiole collapse, usually in Fenjiao; B. Leaf yellowing; C. Stem cracking; D. Browning and necrosis of corms in cross section.

GCTCV-119 seemed resistant to fusarium wilt in the planting cycle in Panyu of Guangdong. This clone came from Taiwan Banana Research Institute, and it was considered of intermediate to high resistance level (Tang and Hwang 1999). In October 2003, 44 suckers of planting cycle were collected and replanted in the neighboring plot for further evaluation of fusarium resistance and agronomic characteristics. External and internal symptoms of fusarium wilt were observed after August of 2004. There were twelve diseased trees as of November 2004. Trees with no wilt symptoms began to flower since mid September, with 11 trees flowered before early November. Average plant height in the second cycle was 2.57 m.

Most of the trees in Dongguan trial plots died of fusarium wilt diseases before shooting or harvesting during the year of 2003. Further evaluation for those cultivars with a few living trees is underway.

In order to screen the true resistant clones, suckers of resistant GCTCV-119 clones in the planting cycle were marked and meristems were cultured in vitro for rejuvenation. Plantlets were planted in farmers' field in April, June, July and August 2004, to find out best planting season and optimize the field management for growth improvement. Preliminary results showed good tree structure, leaf arrangements and

growth vigour from tissue-cultured trees. The total number of trees is more than 1000, which can be the materials of further selection.

Agronomic characteristics

In the planting cycle, complete agronomic data were gained in a few clones. Trees of 'Gros Michel' and 'Yangambi km 5' did not flower in the first year. No fruit reached mature stage before being infected with disease in 'Baxijiao,' 'Williams,' FHIA-17, FHIA-18 and FHIA-23 (Table 3). Trees shot between 310 and 400 days after planting in the planting crop cycles (Table 3). FHIA-03 had the shortest growth cycle, shooting 300 days after planting (range 290 and 330 days), and the fruit maturing at 388 days after planting (range 307 to 472 days).

GCTCV-119 started to shoot 397 days (range 389 to 406 days), and fruits mature 510 days after planting. Its yield, bunch and finger shapes were acceptable and eating quality was very good. However, one or two small leaves could be seen in a tree during early spring, indicating its sensitivity to chills. Attempts are being tried to improve its growth through adjustment of planting time and optimization of field management.

Table 3. Agronomic characteristics of 12 clones in the planting cycle of the IMTP-3 fusarium trial in Panyu, Guangdong Province, China. Planting date: 17 August 2002.

Clone	Days from planting to shooting	Plant crop cycle (days)	Plant height at shooting (cm)	Bunch weight (kg)	No. of hand	Fruit number at harvest	No. functional leaves at flowering	No. functional leaves at harvest
FHIA-02	363.7±21.0	516.0			6.0±1.4	80.0±22.6	13.6±0.7	6.5±0.7
FHIA-03	314.7±21.5	388.7±82.5	256.5±19.1	11.7±8.7	6.0	88.0±5.6	12.8±0.2	9.3±1.2
'Williams'	323.1±8.1						13.9±0.4	
cv. Rose	314.1±29.3	516.0	205.3±13.6		8.7±0.6	116.0±17.4	10.6±1.9	5.3±1.1
FHIA-17	364.0±18.4						14.0±0.0	
FHIA-23	394.0						13.0	
GCTCV-119	397.8±12.4	510.0	227.8±5.6	13.9±2.7	6.4±0.5	99.4±11.5	13.5±0.7	6.2±1.6
SH3436-9	395.3±12.1	501.5±20.5	270.3±11.5	6.1±2.3	8.6±0.9	155.5±3.5	12.3±0.6	2.2±0.2
TMBx5295-1	382.5±16.3	516.0	312.5±0.0	-	6.0	70.0	8.4±7.3	4.5
SH 3640	338.9±11.4	428.7±51.3	250.0	12.3±8.1	8±1.4	112.0±26.9	14.6±1.4	7.0±4.2
FHIA-18	342±13.89						13.3±0.6	
FHIA-21	355.4±19.6	451.5±91.2	281.0		6.5±0.7	71.0±14.1	12.5±0.7	8.0
CRBP 39	306.6±11.9	501.5±20.5	259.4±68.8	5.8	10.3±2.3	143.5±36.1	11.0	4.9±1.2
'Baxijiao'	333.5±3.0						13.7±0.2	

SH3436-9 also had a long growth stage, shooting 395.3 days after planting, whereas CRBP 39, cv Rose and FHIA-03 started to shoot around 300 days after planting, with shorter growth stages.

Crop cycles ranged between 400 and 500 days. FHIA-03 was the shortest (388.7 days). FHIA-02, cv Rose and TMBx5295-1 needed 516 days, while GCTCV- 119, SH 3436-9 needed more than 500 days.

Most of the trees had stems of 200 and 300 cm tall, while TMBx 5295-1 was the tallest at 312.5 cm at shooting, and cv Rose was the shortest at 205.3 cm.

Fruits were harvested only in five clones (Table 3). The biggest bunch was collected in GCTCV-119, with 13.9 kg. No fruit could be harvested in other trees because of poor growth or disease or typhoon damages.

Hand numbers were between 6 and 8 and total fingers 36 and 155. SH 3436-9 had 155.5 while CRBP 39 had only 36.1 fingers. Fingers of SH 3436-9 were small and underdeveloped. Leaf numbers ranged between 10 and 14 at shooting stage and 4 to 9 at harvest. However, SH 3436-9 had only 2.1 leaves at harvest.

One hundred seventy out of 288 trees survived in the second cycle, which did not mean that they were all resistant to fusarium wilt disease since suckers of some susceptible clones may live for two or more years. More observation has been done in the second cycle (Table 4). 'Gros Michel,' Yangambi km 5,' FHIA-23 and SH-3640 still did not shoot, and no mature fruit bunch was harvested in FHIA-17, GCTCV-119 and SH3436-9.

Three 'Williams' ratoon trees were alive and its fruits fully developed. Yet its short stems (1.86 m vs normal 2.0m~4.0m, Daniells 1995), as recorded in earlier report (Orjeda *et al.* 1999), were obviously somaclonal off-types from long-term conservation. 'Williams' is the reference cultivar for FOC susceptibility. Due to many subcultures and long-term conservation, somaclonal variation may be possible. Suckers were collected and in vitro cultured for further evaluation.

Fingers of FHIA-02 were short and thick and with soft flesh. Fruit bunch of FHIA-03 was big and compact. Although its fruit bunch mature early and eating quality was good, cv Rose trees were short and slender, with loose hands and less superior appearances.

Fifteen trees of GCTCV-119 survived in the second cycle, and five trees flowered in the first and second plots. Its leaf shealths were arranged compactly and closely, which may be the main reason for 'Choke throat' when bunch emerged.

The tree stature of 'Gros Michel' was tall and erect. Even though they seemed healthy, they did not flower in the second cycle. A few trees of SH3436-9 flowered and set upward bunches with small fingers.

Three trees of 'Baxijiao' flowered in the ratoon crop. However, all leaves turned yellow and wilted before fruit matured. Therefore fingers were not fully mature when harvested. Normally, the bunch weight of 'Baxijiao' is over 20 kg. It seemed that suckers of 'Baxijiao' might live for one to two years.

Conclusion

Before the trial, pathogen samples taken from the vascular bundles of diseased plants were sent to Australia for identification. VCG 01213 and 01216 were found in these samples, which belong to the tropical race 4 (N. Moore, personal communication). Therefore, the trial should be interpreted as the reaction of banana clones to race 4 of fusarium wilt.

According to the number of trees that survived 12 months after planting, in combined with external symptoms and vascular discolouring, the tested cultivars can be classified as:

Susceptible: 'Williams,' FHIA-17, 'Gros Michel,' 'Baxijiao.' Less than 8 trees survived, over 40 % with external symptoms, or discolouration index over 3.0. All the cultivars tested in Dongguan were susceptible ones.

Table 4. Agronomic characteristics of 12 clones in the 2nd crop of the IMTP-3 fusarium trial in Panyu, Guangdong Province, China.

Clone	No. of trees	Flowering to harvest (days)	Plant height (cm)	Girth (cm)	Bunch weight (kg)	No. of hands	No. of fingers	No. of leaves, flowering	No. of leaves, at harvest
FHIA-02	7	93.5	277.7	52.3	13.2	7.8	109.4	11.9	9.5
FHIA-03	8	86.0	288.0	61.4	12.1	6.4	87.4	10.3	8.8
'Williams'	3	105.7	186.3	48.3	13.6	8.3	130.0	12.3	10.0
cv. Rose	9	84.0	180.1	25.4	6.4	8.2	60.1	8.8	5.1
FHIA-17	1		296.0	67.0		9	135.0	6.0	
GCTCV-119	15		256.5	51.5					
SH 3436-9	1		283.0	65.0				11.0	
TMBx5295-1	8	80.5	307.6	46.9		6.1	74.7	11.3	6.0
FHIA-18	6	81.0	263.7	54.9	10.6	7.7	155.5	11.3	6.0
FHIA-21	10	73.0	306.2	45.5	9.6	6.3	76.1	11.2	7.0
CRBP 39	7	80.0	251.4	38.4	4.8	7.8	101.0	9.6	5.6
'Baxijiao'	5	69.0	280.0	46.4	10.9	7.3	115.5	12.0	2.0

Intermediate: CRBP-39, TMBx5295-1, FHIA-17, FHIA-18, SH-3640, SH3436-9. Eight to twelve trees survived, 25 to 40 % with external symptoms, or vascular discoloration index 1.5 to 3.0.

Resistant: FHIA-02, FHIA-03, cv Rose, FHIA-23, GCTCV-119, FHIA-21. More than twelve trees survived, 25 % with external symptoms, or discoloration index below1.5.

In other regions, bunch weight of GCTCV-119, the Cavendish clone developed by Taiwan Banana Research Institute (TBRI) through selection from somaclonal variants, ranged from 3.3 and 22.2 kg, average crop cycle was 533 days, with hand number of 6.67 and plant height of 2.51 m (Orjeda *et al*. 1999). Although in some sites it did not show good resistance to fusarium wilt like in Taiwan (Orjeda *et al*. 1999), it performed well in Panyu, probably attributing to the similar genetic background of the pathogen. Its fruit quality was very good and yield was acceptable. However, one shortcoming was its long cropping time. Strong suckers or tissue-cultured plantlets are encouraged as planting materials, and planted in deep, fertile soils. Extra nitrogen and potassium fertilizers may be needed, in order to stimulate tree growth. GCTCV-119 was sensitive to low temperatures in winter and early spring. A few short and narrow leaves were seen in spring. 'Choke throat' was seen in a few trees. Change of planting time would be effective in order for trees to grow in warm season. Production of virus-free plantlets, screening of new good strains or improvement through induction are also attempted.

'Williams' has been a cultivar being easily subjected to somaclonal variation, especially those buds under long-term storages (Orjeda *et al.* 1999). Therefore, variation with resistance or tolerance to Foc race 4 may be expected. In vitro culture has been established for further observation.

Some of the improved FHIA hybrids showed high level of resistance. However, the texture and flavor of the fruit are not acceptable to the consumers in China who are accustomed to the flavor of Cavendish, 'Xiangjiao' or 'Fenjiao.'

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