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Somaclonal Resistance in Cavendish Banana to Fusarium Wilt

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ABSTRACT

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Somaclonal variants of Giant Cavendish derived from meristem culture were used for screening for resistance to fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* Race 4. As a result of this screening program, 9 somaclones showing resistant reaction had been obtained. Four of them （GCTCV-44, -104, -105, -119）were highly resistant to the disease, while the others （ GCTCV-46, -53, -62, -201, -215 ） were moderately resistant. Somaclones with high level of resistance had a percentage of disease incidence of less than 10% and those with moderate level of resistance had 11-30%, compared with over 70% in Giant Cavendish. All resistant clones differed from the parent, Giant Cavendish, by possessing combinations of inferior agronomic characters. Improved variants were obtained from a large planting in the field of plantlets of these resistant clones. The majority of improved variants retained wilt resistance. GCTCV-215-1 appears to be the most promising resistant variety because of having both acceptable horticultural characteristics and marketable fruit qualities. Disease incidence of GCTCV-215-1 was 17.2% when 2-month-old plantlets were used as planting material and it was 5.2% for suckers, compared with about 75% in Giant Cavendish. Plantlets of GCTCV-215-1 had been planted in 700 ha of infested banana fields during the period of March-May 1990 for further evaluation of the stability of disease resistance under a wide range of environmental conditions.

（Key words : banana, Fusarium wilt, disease resistance）

INTRODUCTION

The Cavendish cvs. Giant Cavendish and Robusta have been seriously affected by Race 4 of *Fusarium oxysporum* Schlect. f. sp. *cubense* (E. F. Smith) Snyder and Hansen in Taiwan in recent years[1] Damage by the pathogen first appeared in 1967 and since 1977 has become widespread in the major banana-production areas in the country, destroying over 500 ha annually[13]. Outbreaks of fusarium wilt on Cavendish cultivars have also been reported from Australia, South Africa, the Canary Islands and the Philippines[7,8,11]. Prior to 1960, fusarium wilt of banana, caused by Race 1 of the pathogen, destroyed more than 40,000 ha of bananas in Central and South America over a period of 50 years[9]. The disease has been under control there since the susceptible cv. Gros Michel was replaced with resistant Cavendish cultivars. Race 2, capable of attacking cv. Bluggoe and Race 3, which attacks Heliconia species, are of minor importance[9].

Previous control measures in Taiwan which consisted of field sanitation, soil fumigation and liming, were not successful[1] Although crop rotation with paddy rice has been shown to be an effective method for controlling the disease[1], replanting of banana in the reclaimed infested fields allowed only one to two years of profitable banana production before the disease increased to epidemic levels. Therefore, it is necessary to develop resistant cultivars to replace the present susceptible Cavendish cultivars.

All commercial banana cultivars tested previously in Taiwan were susceptible to Race 4 of F. *oxyspomm* f. sp. *Cubense*[4] Searches for a resistant mutant were made among survivors in fields of Cavendish cultivars devastated by wilt, but without success[13] Since about 3% of the plantlets derived from tissue culture of Giant Cavendish are mutants[2], these plantlets were screened for resistance to Race 4 beginning in 1984.

MASS PROPAGATION OF PLANTLETS AND SCREENING PROCEDURES

The method of Ma and Shii[6] was used to induce the formation of adventitious buds from explants obtained from shoot tips of suckers of Giant Cavendish. Subculture of the buds was made once a month and continued 6-7 times until a sufficient population of buds was achieved. Procedures described by Hwang *et al*[3] were used to induce roots and promote shoot development from the adventitious buds. Plantlets were maintained in a green-house for 2 months before planting in a field in which about 60% of the Cavendish plants had shown wilt symptoms. After diseased tissue had been plowed into the soil, a pathogen population of about 300 to 1,000 propagules per gram of soil was maintained throughout the period of testing. A large number of 2-month-old plantlets were planted in the nursery, giving a planting density of about 20,000 plants per ha. After 3-4 months, the surviving plants were dug up and the rhizomes were cut and examined for symptoms of fusarium wilt. Those free of symptoms were multiplied by shoot-tip culture for further tests. When suckers of the surviving plants were used for planting, rhizomes were examined for symptoms by shaving with a knife[13]; only disease-free suckers were tested.

Tissue-culture-derived plantlets have been propagated commercially since 1982 for the banana planting program in Taiwan[3]. A few banana plantations which were established with these plantlets became infested with the wilt pathogen after 2-3 years. Suckers of survivors in seriously diseased plantations were also selected and multiplied in vitro for resistance testing.

RESISTANT MUTANTS OBTAINED FROM PLANTLETS

Of about 20,000 plantlets screened thus far, six have been found to be highly resistant to the pathogen（Table 1）. After one year, all mature plants grown from suckers of the six remained healthy, while 60% of the susceptible control plants were diseased. Resistance of the six variants remained high during a second and third generation, regardless of whether plantlets or suckers were used to propagate the plants. Results of a greenhouse experiment indicated that all six clones were resistant to Race I, as well as Race 4 of *F.*

oxysporum f. sp. *cubense* and hence there is no evidence that there is a breakdown of resistance to Race I during in vitro manipulation.

The resistance of these clones is affected by the inoculum density of the pathogen in soil. Results of an artificial inoculation test showed that at inoculum densities of 300 propagules per g of soil, all plantlets of the resistant clones GCTCV-53 and GCTCV-119 remained healthy under greenhouse conditions, while 65% of the plantlets of the susceptible parent were killed. To cause an incidence of 50% disease in the parent, GCTCV-53 and GCTCV-119, inoculum densities of 100, 850 and 1,000 propagules $soil^{-1}$ were required, respectively. GCTCV-119 appears to be more resistant than GCTCV-53.

All six resistant clones differed from the parent. Giant Cavendish, by possessing combinations of inferior agronomic characters such as excessive height, weak petiole, small fruit bunches and lengthened growth period（Table 2）.

Table 1. Incidence of fusarium wilt in six tissue culture-derived variants of Giant Cavendish[1)]

Clone	Planting material	Number evaluted	% Diseased
GCTCV-40	Sucker	329	0.5
	Plantlet	965	0.3
GCTCV-44	Sucker	118	2.0
	Plantlet	250	0
GCTCV-46	Sucker	268	1.8
	Plantlet	596	0.5
GCTCV-53	Sucker	184	0.2
	Plantlet	467	3.9
GCTCV-62	Sucker	40	3.0
	Plantlet	45	11.1
GCTCV-119	Sucker	75	0
	Plantlet	650	2.2
Giant	Sucker	207	39.5
Cavendish	Plantlet	863	59.7

1）Average disease incidence for 3 generations of each clone. The variants and Giant Cavendish were screened in a field naturally infested with Race 4 of *fusarium oxysporum* f. sp. *cubense*.

Table 2. Major agronomic characteristics of resistant clones derived from tissue culture of Giant Cavendish relative to the normal parent

Clone	Salient features
GCTCV-40	Taller, thinner pseudostem with wide spacing of internodes; weak petioles; narrow, drooping leaves（resembles tetraploid）
GCTCV-44	Shorter, thinner pseudostem, weak petioles and drooping leaves; normal bunch but with weak finger neck（resembles tetraploid）
GCTCV-46	Black spots on pseudostem, petiole and leaf sheaths; more upright leaves; smaller bunches with short fingers
GCTCV-53	Dark green pseudostem and leaves; drooping leaves; male bud narrow and elongate; smaller bunches with short fingers
GCTCV-62	Pale green pseudostem and leaves; slow to produce suckers; smaller bunches and fingers
GCTCV-119	Taller, thinner pseudostem; wavy lamina with pronounced purple spotting; rose shades in pseudostem, petioles and leaf sheaths when young; loosely-packed pseudostem at maturity

IMPROVEMENT OF RESISTANT MUTANTS

All six resistant clones had no commercial value because the fruit was under the minimum standard of quality. Selections for mutants with commercially acceptable fruit were made from tissue-culture progenies of the resistant clones（plantlets of resistant clones were propagated by the same method as described above）. When large numbers of plantlets of the resistant clones were planted in the infested field, a few plants with better fruit quality were found in each clone（Table 3）. These plants had thicker pseudostems, grew faster and produced a bigger bunch than their respective resistant parents. The percentages of improved variants found in plantlets of these six clones ranged from 0.2% to 10.1%.

Of four variants tested, GCTCV-44-1, GCTCV-53-1 and GCTCV-119-1 were highly resistant to Race 4, while GCTCV-40-1 had lost wilt resistance（Table 4）. In a large scale field trial involving a total of 10 ha of plantings at various locations, the incidence of disease in GCTCV-44-1, GCTCV-53-1 and GCTCV-119-1 averaged I.I, 6.3 and 3.9%, respectively, compared to 60.9% in Giant Cavendish.

The resistant variants yielded much heavier bunches than those produced by the original resistant clones（Table 5）. For instance, the weight per bunch on GCTCV-119 plants was 17.2 kg, while that of its variant reached 26.5 kg, an increase of about 54%. The bunch weights of GCTCV-44-1 and GCTCV-119-1 were about the same as those of normal plants, while GCTCV-53-1 produce a bunch about 17% lighter than normal. All the fruit produced by these three resistant variants ripened normally upon ethylene treatment.

In appearance, mature plants of the three improved resistant variants are distinct from the parental clones. GCTCV-119-1 takes longer to produce fruit and is taller than Giant Cavendish, whereas GCTCV-44-1 has weak petioles and drooping leaves, characteristics normally associated with tetraploids. These agronomic characters are considered to be deficiencies in banana plants. Although mature plants of GCTCV-53-1 were 30 cm shorter than those of Giant Cavendish, a desired character, they produced hands with shorter fingers. A search for resistant banana plants that lack agronomic defects is neeeded.

Table 3. Frequency of variants with improved fruit quality among plantlets from tissue-cultun derived resistant clones

Clone	Number of plants evaluated	Improved variants	
		Number	Frequency（%）
GCTCV-40	1,040	105	10.1
GCTCV-44	1,219	3	0.2
GCTCV-46	592	8	1.3
GCTCV-53	220	12	5.5
GCTCV-62	45	2	4.4
GCTCV-119	45	4	8.9

Table 4. Disease incidence in improved, wilt-resistant variants of Giant Cavendish

Clone	Planting material	Number evaluated	Disease incidence[1)]
GCTCV-40-1	Sucker	47	57.1
	Plantlet	45	44.4
GCTCV-44-1	Sucker	81	3.2
	Plantlet	219	4.5
GCTCV-53-1	Sucker	300	4.7
	Plantlet	118	6.2
GCTCV-119-1	Sucker	376	3.1
	Plantlet	900	4.8
Giant	Sucker	157	50.9
Cavendish	Plantlet	150	63.3

1）Means of two trials in separate fields.

Table 5. Bunch weights for resistant clones, variants of the clones and Giant Cavendish

Clone	Bunch weight（kg）[1)]	
	Original	Variant
GCTCV-44	18.6 b	25.0 c
GCTCV-53	13.8 b	21.5 c
GCTCV-119	17.2 b	26.5 c
Giant Cavendish	25.9	

1）Bunch weights are means for 50 plants of each clone and its variant harvested in June and July. Means in rows followed by the same letter are not significantly different(P> 0.05)according to Duncan's multiple range test. Mean bunch weight for Giant Cavendish is significantly greater than that for the variant of GCTCV-53, but not for the variants of GCTCV-44 and GCTCV-119.

A PROMISING RESISTANT VARIANT FOR CONTROLLING FUSARIAL WILT

Recently, a new improved variant, GCTCV-215-1, with agronomic characters superior to the three clones mentioned above was obtained from the tissue culture plantlets of Giant Cavendish. The variant had a desirable level of resistance to fusarial wilt. Disease incidence of GCTCV-215-1 was 17.2% when 2-month-old plantlets were used as planting material and 5.2% when suckers were used for planting, compared with about 75% in Giant Cavendish（Table 6）. Compared with Giant Cavendish, the variant was slightly taller, had a thinner pseudostem and took about one month longer from planting to harvest（Table 7）. When mature, the leaves of this variant usually became necrotic on the margin, forming a brown or grey band of 1-2 cm wide along both sides of leaf margin. This distinctive feature of GCTCV-215-1 easily distinguishes it from Giant Cavendish. The variant yielded 28 kg per bunch which is about the same as that for Giant Cavendish. Fruit produced by the variant ripened normally upon ethylene treatment and had a shelf-life and flavor similar to that of Giant Cavendish. Because of having both acceptable agronomic characters and marketable fruit quality, GCTCV-215-1 appears to be a promising resistant variant for commercial planting for controlling fusarial wilt.

Table 6. Disease incidence in GCTCV-215-1 and Giant Cavendish

Clone	Planting material	Number evaluated	Disease incidence[1)] (%)
GCTCV-215-1	Sucker	427	5.2
	Plantlet	1,881	17.2
Giant Cavendish	Sucker	560	77.8
	Plantlet	1,647	74.6

1）Means of 10 and 5 trials in separate fields for plantlet and sucker, respectively.

Table 7. Major agronomic characters of improved resistant clones of Giant Cavendish

Clone	Plant ht. (cm)	Circumference of pseudostem (cm) [1)]	Growth cycle (mo)	Bunch weight (kg) [2)]
GCTCV-44-1	260	66	12-13	25.0
GCTCV-53-1	257	75	11-12	21.5
GCTCV-119-1	333	83	14-15	26.5
GCTCV-215-1	286	74	13-14	28.0
Giant Cavendish	277	81	12-13	27.7

1）Measured at 30 cm above ground.
2）Average of 50 plants for each clone harvested in May and June.

DISCUSSION

Race 4, the cause of fusarium wilt of Cavendish bananas, is more destructive and has a wider host range than Race 1[(4)]. Finding a resistant clone to replace the susceptible Cavendish cultivars is urgently needed. Traditional banana breeding for improvement, including resistance to fusarium wilt, has been based on obtaining useful tetraploids from crosses between triploids and diploids. However, due to low fertility inherent in most triploids and a lack of outstanding, fertile parents, banana breeding during the last 60 years has met with very limited success. Stover and Buddenhagen[(10)] have discussed, in detail, the reasons for the failure of previous breeding efforts to obtain useful resistant varieties.

Since bananas have been extensively cultivated on a large scale for a long time, it is commonly assumed that a substantial number of somatic mutants exist in nature. In a selection program to determine if a resistant mutant could have arisen from the local susceptible Cavendish cultivars in Taiwan, a large number of suckers from healthy plants which were growing in wilt-devastated fields were tested against Race 4 of *F. oxysporum* f. sp. *cubense*. No resistance to the pathogen was found[(13)]. Similarly, searches for a resistant mutant of the wilt-susceptible Gros Michel made in many countries in tropical America

during the 1920s were not successful[9]. These results suggest that genetic variation occurring during the vegetative reproduction of cultivated bananas is rare under plantation conditions, and that the chance of obtaining a wilt resistant mutant in this manner is extremely small. In Taiwan, no significant variation was found in 40,000 mature plants grown from suckers in commercial plantations[2].

In contrast, great variability and high level of resistance to fusarium wilt were found in some clones of banana derived from *in vitro*-generated populations and subsequently propagated via shoot-tip culture[2,5]. Although all 6 resistant mutants originally obtained from the screening program were morphological variants producing fruit with poor quality[5], a few plants with improved fruit quality were found in their subsequent *in vitro*-derived progenies. The improved variants of GCTCV-44, GCTCV-53 and GCTCV-119 remained highly resistant to fusarium wilt. When these improved variants were planted in infested fields at various locations in Taiwan, their resistance to fusarium wilt was found to be stable over a wide range of environmental conditions.

The genetics of resistance of fusarium wilt of banana is not known. Race 4 can attack cvs, Gros Michel, Bluggoe, Pisang lilin and Latundan in addition to Cavendish cultivars[12]. The mutants derived from tissue culture of Giant Cavendish are resistant to both Race 1 and Race 4. but their reactions to other races remain to be tested. A susceptible segregant was found amongst the variants of the resistant clone GCTCV-40, which has narrow, drooping leaves. The susceptible revertant has erect leaves characteristic of normal plants. In contrast the variants of GCTCV-44, GCTCV-53, GCTCV-119 and GCTCV-215 which retained the atvpical foliage appearance, remained wilt resistant. This suggests that the gene（s）conferring resistance to Race 4 in these mutants may be linked to that（those）governing certain foliage characters. By continuous planting of a large number of tissue-culture-derived plantlets of resistant mutants（themselves of in vitro origin）in infested fields, it may be possible to determine the genetic relationship between susceptibility and morphological characteristics.

The commercial value of the resistant variants depends on the productivity and fruit quality of these clones relative to the Cavendish cultivars which are now used by the international banana export trades. Among the resistant

variants obtained thus far, GCTCV-215-1 is the best. It resembles Giant Cavendish except that it is slightly taller and has a longer growth cycle（Table 7）. Plantlets of this variant were planted in about 700 hectares of infested banana fields during the period of March-May 1990 for further evaluation on the stability of disease resistance under a wide range of environmental conditions. Further selection to obtain a superior resistant variant from plantlets of GCTCV-215-1 in widespread planting is in progress.

LITERATURE CITED

1.Hwang, S. C. 1985. Ecology and control of fusarial wilt of banana. Plant Prot. Bull.（Taiwan）27:233-245.
2.Hwang, S. C. 1986. Variation in banana plants propagated through tissue culture.（In Chinese with English summary）. J. Chinese Soc. Hort. Sci. 32:117-125.
3.Hwang, S. C., Chen, S. L., Lin, J. C., and Lin, H. L. 1984. Cultivation of banana using plantlets from meristem culture. Hort Science 19:231-233.
4.Hwang, S. C., Chen, C. L., and Wu, F. L. 1984. An investigation on susceptibility of banana clones to fusarial wilt, freckle and marginal scorch disease in Taiwan. Plant Prot. Bull.（Taiwan）26:155-161.
5.Hwang, S. C., and Ko, W. H. 1988. Mutants of Cavendish banana resistant to race 4 of *Fusarium oxysporum* f. sp. *cubense*. Plant Prot. Bull.（Taiwan）30:386-392.
6.Ma, S. S., and Shii, C. T. 1972. *In vitro* formation of adventitious buds in banana shoot apex following decapitation.（In Chinese with English summary）. J. Chinese Soc. Hort. Sci. 18:135-142.
7.Mayer, P. E. 1983. Fusarium wilt of Cavendish bananas in Queensland.（Abstr.）Proc. Int. Fusarium Workshop 5:60.
8.Philips, D., and Dale, J. 1983. Isozyme and soluble protein techniques for identification of races of the banana wilt pathogen in Queensland.（Abstr.）Proc. Int. Fusarium Workshop 5:76.
9.Stover, R. H. 1962. Fusarial Wilt（Panama Disease）of Banana and other Musa Species. Phytopathol. Pap. 4. Commonw. Mycol. Inst., Kew, Surrey, England, 117p.

10.Stover, R. H., and Buddenhagen, I. W. 1986. Banana breeding: Polyploidy, disease resistance, and productivity. Fruits 41:175-191.
11.Stover, R. H., and Malo, S. E. 1972. The occurrence of fusarial wilt in normally resistant 'Dwarf Cavendish' banana. Plant Dis. Rep. 56:1000-1003.
12. Su, H. J., Chuang, T. Y., and Kong, W. S. 1977. Physiological race of fusarial wilt fungus attacking Cavendish banana of Taiwan.（In Chinese）Taiwan Banana Res. Inst. Spec. Publ. 2,21pp.
13. Su, H. J., Hwang, S. C., and Ko, W. H. 1986. Fusarial wilt of Cavendish banana in Taiwan. Plant Dis. 70:814-818.

摘要

黃新川 1991 **華蕉體細胞變異之抗黃葉病選種** 植保會刊 33:124~132（屏東縣台灣香蕉研究所)

從北蕉（Giant Cavendish）之組織培養變異品系（Somaclonal variant）篩選已獲得 9 個品系對香蕉黃葉病病原菌（*Fusarium oxysporum* f. sp. *cubense*. race 4）具有抗病性，其中 4 個品系（GCTCV-44, -104, -105, -1T9）呈高度抗病性，於重病蕉園測定其平均發病率低於 10%；5 個品系（GCTCV-46, -53, -62, -201, -215）呈中度抗病性，其平均發病率介於 11~30%之間；對照一般北蕉之發病率為 70%以上。所有抗病品系均帶有不良的園藝變異性狀，從其大量種植之後代植株中可尋獲改良型品系。大多數改良型品系仍維持與原母系相同的抗病程度。田間試種結果顯示改良聖品系之中以 GCTCV-215-1 之園藝性狀及香蕉產量品質較為理想，具有推廣種植潛力。在重病蕉園種植 GCTCV-215-1 組織培養苗之發病率為 17.2%，吸芽苗之發病率為 5.2%，對照一般北蕉之發病率為 75%。79 年 3~5 月期間於本省中南部香蕉黃葉病區種植 GCTCV-215-1 之組織培養苗 700 公頃，俾進一步探討其種植在不同環境條件下之抗病穩定程度。

（關鍵字：香蕉、香蕉黃葉病、抗病）