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BREEDING OF CAVENDISH BANANA FOR RESISTANCE TO FUSARILM WILT BY SOMACLONAL VARIATION APPROACH

Shin-Chuan Hwang

Taiwan Banana Research Institute, P.O.BOX 18, Chiuju Pingtung, Taiwan 904,

R. O. C Email : tbri@ksts.seed.net.tw

ABSTRACT

Fusarium wilt of banana, caused by *Fusurium oxysporum* f. sp. *cabense* race 4, is one of the major constraints for cultivation of banana in Taiwan. To prevent the spread of disease by planting material, technology of mass propagation of disease-free tissue cultured plantlets and of using plantlets for commercial banana production was successfully developed in 1983 by the Taiwan Banana Research Institute. These tissue cultured plants showed up to 3 per cent somaclonal variation, thus offering an alternative for banana improvement. A mass-screeing program based on somaclonal variation for detecting resistance to Fusarium wilt race 4 was therefore set up by planting the *in vitro* produced plants in a nursery heavily infested with diseased tissue. This breeding program, begun 1984, has produced a total of 13 resistant clones so far, all derived from the wilt-susceptible variety Giant Cavendish. Two of them have been released as the new varieties, Tai-*Chiao No. 1* in 1992 and *Tai-Chiuo No. 3* in 2001. For commercial production. They are now planted on over 1,600 ha of Fusarium-in tested farms, Moderate success has been obtained for both varieties are only mediocre in resistance to Fusarium wilt and in productivity. More recently, a really good substitute for the *Giant Cavendish* was obtained. This new clone, *GCTCV-218*, was highly resistant to Fusarium wilt and produced a bunch about 42% heavier than that produced by its parent Giant Cavendish, a breakthrough in banana breeding. It is scheduled for release as the new variety, *Formosana*, for replacing both *Tai-Chiao No. 1* and T*ai-Chiao No. 3* in 2002. The innovative breeding strategy based on somaclonal variation has proved to be an efficient and useful method for the improvement of banana.

Key words: banana, breeding, tissue culture, somaclonal variation, Fusarium wilt, disease resistance

INTRODUCTION

In Asia, the oldest international banana trade exists in Taiwan where banana production for exporting to the neighboring Japanese market began in the early 1900's. The banana industry, involving small producers mostly, expanded rapidly and reached the peak production on over 50,000 hectares in the mid-1960's, ranking Taiwan the second largest banana exporting country in the world. Under the subtropical climate, bananas produced in Taiwan have top eating quality, and they are highly esteemed in the Japanese market.

Over the last 20 years, production of Cavendish banana in Taiwan, however, has been seriously jeopardized by the intractable Fusarium wilt problem[2,14]. The fungus attacking Cavendish, previously considered highly resistant to this disease, belongs to the type of race 4 of *Fusarium oxysporum* f. sp. *Cubense*[13]. The disease, found in 1967, has spread rapidly to the epidemic proportion within few years and caused considerable losses to the banana growers. At present, about 4,000 of the total 6,000 hectares of banana growing in the central and southern part of the island are affected. Attempts to controlling the disease using soil sanitation, soil fumigation, and soil amendment, etc. were ineffective[2,14]. A commercially acceptable resistant variety is, therefore, urgently needed.

Banana breeding based on the traditional crossing (pollinating) approach is extremely difficult, for Cavendish especially, because of the female seed sterility of the Cavendish varieties. Seventy years of crossing a short *Gros Michel* commercial banana with a resistant diploid to obtain a resistant tetraploid competitive with the Cavendish cultivars has been unsuccessful[12]. For this reason, for obtaining a Fusarium wilt resistant Cavendish, an innovative breeding approach based on somaclonal variation was taken by the Taiwan Banana research Institute (TBRI) in 1984. This paper gives an account of the progress this breeding program has made with highlight on the development of a super high-yielding, Fusarium resistant clone which upon commercialization could have great impact on Taiwan banana industry.

IN VITRO PROPAGATION AND SOMACLONAL VARIATION

Although in vitro propagation of vegetatively propagated crops such as banana has shown potential for producing substantial genetic variability, known as "somaclonoal variation" [15],it is generally believed that the probability of success in obtaining an improved Cavendish clone that also corrects its susceptibility to Fusarium wilt race 4 based on somaclonal variation would be extremely low, if not impossible. The key to the success is to produce a large number of seedlings in vitro for screening. Therefore, the development of a tissue culture program for efficient, cheap production of seedlings is the most crucial First step. It is also important that the seedlings must be virus-free.

Technology of mass propagation of banana tissue cultured plantlets and of using plantlets for commercial banana production was successfully developed in 1983 by TBRI[1], The procedure for commercial propagation of banana tissue cultured plantlets consists of culture initiation, bud multiplication, plantlet regeneration and acclimatization.

The method of Ma and Shii (1972) was adopted for inducing adventitious buds by using Murashiege and Skoog medium supplemented with BAP (6-bcnzylaminopurine) as a cytokinin at a concentration of 0.5 mg/l[11], Suckers selected from healthy and true-to-type mother plants grown in the field were indexed for Banana Bunchy Top Virus, Cucumber Mosaic Virus and Banana Streak Virus, the 3 viral diseases of banana present in Taiwan[9]. Those showing virus-free were maintained in an insect-proof, vector-free screenhouse as the foundation stock from which new suckers developed were used for tissue culturing purpose. For culture initiation, a small block (1 cm^3) of meristematic tissue was removed aseptically from the sucker to induce adventitious bud formation. Shoot/bud clusters were subcultured during the multiplication stage for 6 to 7 cycles. Shoots were regenerated into plantlets by adding a liquid medium into the bottles of final subculture. Finally, plantlets were transplanted into a soilless potting mix and acclimatized in the screened nursery for 2 months before field planting.

The initiative of the development of the tissue culture program at TBRI came from the Fusarimm wait. As a systemic disease, it spreads readily through the movement of infested suckers, the conventional planting material used by farmers[2,14], The adoption of disease-free plantlens by farmers has proved useful to check the spread of the disease. They also have the advantaiges or having a higher survival rate than suckers, lower cost in disease and pest control, and a shorter harvesting period because of the uniform and vigorous growth[1]. Field surveys revealed that these plants showed up to 3 per cent somaclonal variation[3]. The range of mutants found is shown in Table 1. Some are detectable when plants are young and others after flowering. Viost somaclonal variants were genetically stable. Although most mutants carry undesirable agronomic traits and produce under-sized bunches, the genetic variability brought about by *in vitro* propagation offers an option for the improvement of banana cultivars.

The program has mass-produced more than 2 million of disease-free plantlets each year of a Cavendish variety Giant Cavendish, the major variety grown in Taiwan, for use by farmers and for research including breeding.

Table 1. Some somaclonal mutations observed in Taiwan Cavendish bananas mass-produced \n vitro without mutagens

Stature

a) Various degrees of dwarfism ; most mutants have short petioles and leaves, short intemodes, and produce small bunches with short fingers.
b) Giantism
Excessively tall plants with long distance between internodes.

Foliage

a) Drooping leaves, weak petioles, wide spacing of intemodes (resemble tetraploid).
b) Narrow leaves, usually more upright.
c) Irregular shape to lamina, sometimes tattered or a portion missing.
d) Shorter, smaller leaves.
e) Waffled or wavy edges of lamina with changes in leaf thickness.
f) Increased waxiness.

Plant color

a) Darker green or different shades of green.
b) Rose shades in petiole and leaf sheath.
c) Purple to black shades or black spotting in petioles and leaf sheath.
d) Variegated leaves.

Pseudostem characteristics

a) Thicker pseudostem.
b) Thinner pseudostem.
c) Woody texture.

Fruit characteristics

a) Small bunches with shorter fingers.
b) Narrow and elongate male bud.
c) Sweetish flavour.

RESISTANT CLONES IDENTIFIED IN SCREENING PROGRAM

A mass-screening program for detecting resistance to Fusarium race 4, begun 1984, was set up by planting *in vitro*-produced plantlets in a nursery soil heavily infested with diseased tissue[4,6]. The diseased tissue was plowed and thoroughly mixed with soil in order for the pathogen to distribute uniformly, maintaining an inoculum dose ranging from 300 to 1,000 popagules/g soil throughout the testing. Two-month-old plantlets of the wilt-susceptible varieties propagated by the above method were used for screening by planting them at high density (20,000 plants/ha) in the nursery. After 3-4 months, depending on seasonal temperature. the surviving plants were dug up and the rhizomes examined for infection. Those free of infection were again multiplied in vitro for additional tests to confirm resistance.

From 1984 to 1986, about 30,000 tissue cultured plants of Giant Cavendish were screened and ten resistant clones selected, following final evaluation[4,6,7]. From 1992 to 1995, two resistant clones were found among 11,180 tissue cultured plants of *Tai-Chiao No. 2*, a semi-dwarf Cavendish cultivar[10]. The efficiency of selection was about two to three resistant clones for every 10,000 plants screened. Among the ten selected clones derived from Giant Cavendish, five were highly resistant with a disease incidence of less than 10%, while the other five were moderately resistant, with an infection rate of 10-30%, compared to over 70% in Giant Cavendish. The horticultural characteristics of these resistant clones are presented in Table 2.

In addition to the normal screening procedure, work on selection was also conducted on the ordinary commercial farms, in collaboration with farmers. By 1990, the tissue cultured plants had already grown widely on more than half of the banana farms, and many of these farms were invaded by the Fusarium wilt, seriously. On these farms, selection was targeted at those apparent healthy plants occasionally found in disease hot spot, with special attention to those showing morphologically different from the normal Giant Cavendish plant but bearing good bunches. Suckers of these putative resistant plants were collected and further multiplied in vitro for testing against Fusarium race 4. Clones

GCTCV-216, *GCTCV-217* and *GCTCV-218* were selected this way[10].

IMPROVEMENT OF RESISTANT CLONES

With exception of the latest selected clone *GCTCV-218* which has superior agronomic characteristics, all the rest of resistant clones are inferior to their parent Giant Cavendish either in agronomic traits or in yield[5]. As indicated in Table 2, most resistant clones possess undesirable agronomic characters such as excessive height, weak petiole with drooping leaves, and produce under-sized bunch, while others like *GCTCV-216* and *GCTCV-217* produce heavy bunches but having too large hands of the former and too short fingers of the latter, rendering them unacceptable to the market. Selection of improved variant from these inferior resistant clones was attempted, again based on somaclonal approach.

When large numbers of tissue cultured plantlets of resistant clones were planted in the field, a few plants with improved agronomic characters were found in each clone. These improved types had thicker pseudostems, grew faster, and produced bigger bunches than their respective resistant parents. The clone *GCTCV-119* for instance, the bunch weight of the original resistant parent was 17.2 kg only, while that of the improved variant was increased up to 26.5 kg[7]. The growing cycle of the improved variant was also shortened from 15 months of the parent to 13 months only. The frequency of improved variants found in these resistant clones varied from 0.2 to 10.1%[5]. Of considerable interest is that the gene(s)conferring resistance to Fusarium race 4 in most of the resistant clones appears to be transmissible to their respective improved variants. Among these four improved variants tested, *GCTCV-44-1*, *GCTCV-53-1* and *GCTCV-119-1* remained to be resistant to Fusarium race 4, and *GCTCV-40-1* only had lost wilt resistance（Table 3）.

Further work on improvement of these resistant clones each year based on somaclonal variation has led to the continued discovery of many new, useful, resistant improved variants, and to the release of two more productive improved variants for commercial production in 1992 and in 2001, respectively.

Table 2. Horticultural characteristics of Fusarium wilt race 4-resistant clones derived from *Giant Cavendish* by somaclonal variation.

Clone	Resistance	Horticultural characteristics
GCTCV-40	High	Tall and slender pseudostem; weak petiole with narrow and drooping leaves; small bunch
GCTCV-44	High	Short and slender pseudostem; weak petiole and drooping leaves; bunch normal but weak pedice
GCTCV-46	Moderate	Black spots on pseudostem and leaf sheath; upright leaves; small bunch with short-fingers
GCTCV-53	Moderate	Dark green pseudostem; drooping leaves; elongate male bud; small bunch with short fingers
GCTCV-62	Moderate	Pale green pseudostem; fewer suckering; small bunch and fingers
GCTCV-104	High	Pale green pseudostem; fewer fingers; long growing cycle
GCTCV-105	High	Shorter and slender pseudostem; compact bunch with more number of short fingers
GCTCV-119	High	Very tall; wavy leaves; short fruit stalk; long growing cycle; fewer hands but large fingers; sweeter fruit
GCTCV-201	Moderate	Robust pseudostem; short fruit stalk; malformed hands
GCTCV-215	Moderate	Tall and slender pseudostem; leaf tip curl and splitting ;fewer suckering; normal bunch but slender fingers; long growing cycle
GCTCV-216	Moderate	Very tall; very large and heavy bunch; long growing cycle
GCTCV-217	High	Erect leaves; compact, but heavy bunch; less carved fingers
GCTCV-218	High	Robust pseudostem; wider and thicker leaves; very heavy bunch with more number of hands; less curved fingers; long growing cycle

GCTCV, Giant Cavendish tissue-culture variant; High, disease incidence of less than 10%; Moderate, disease incidence of 10-30%.

Table 3. Fusarium wilt on improved variants as compared to their resistant parents

Improved clone	Planting material	Number of plants tested	Disease* (%)
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 Cavendish	Sucker	157	50.9
	Plantlet	150	63.3

*Means of two trials in separate field.

Release of Resistant Clones for Commercial Planting

For the control of Fusasrium wilt in Taiwan, there have been two resistant clones, *GCTCV-215-1* and *TC1-229*, released for commercial production[8,10]. The latest selected clone, *GCTCV-218*, is scheduled for release for commercial planting, beginning January, 2002.

GCTCV-215-1

GCTCV-215-1, a moderately resistant clone, was released in 1992 for commercial production as the new variety *Tai-Chiao No. 1*[8], the first release anywhere of a mutated Cavendish variety. It was selected in 1988 and is the secondary variant of *GCTCV-215*. It is slightly taller than *Giant Cavendish* and has a more slender pseudostem and longer growing cycle. Although the bunch weight of this new variety is about 10% lighter than that of *Giant Cavendish*, it is reputed to have more uniform bunch shape and more even ripening, giving better-quality fruit[8]. It has been estimated that *Tai-Chiao No. 1* is now planted on about 1,500 out of 4,500 ha of banana farms in southern Taiwan every year. Surveys in infested Fields planted with this new variety showed that the percentage of wilt incidence averaged 6.5% in 1994 and 5.1% in 1995. The incidences for Giant Cavendish were 69.0 and 42.6%[7]. The release of *Tai-Chiao No.1* has reduced considerably the yield loss to Fusariurn wilt and enabled growers to resume banana production on many abandoned infested farms.

TC1-229

TC1-229, a semi-dwarf variant of *Tai-Chiao No. 1*, was selected from a farmer's field in 1992[10]. It is about 50-70 cm shorter than its parent *Tai-Chiao No. 1*, Other traits such as growth cycle, bunch weight and level of resistance to Fusarium wilt race 4, were not significantly different from those of *Tai-Chiao No. 7*. Since wind damage is one of the major constraints for cultivation of banana in Taiwan, planting the shorter variety would reduce yield losses and facilitate farm management. For this reason, the clone was released in 2001 as the new variety *Tai-Chiao No. 3* for commercial production. It is now planted on some 100 ha of infested farms.

GCTCV-218

The clone was discovered by a farmer in 1993 on his farm planted with tissue cultured plantlets of Giant Cavendish provided by TBRL He did not report to TBRI until 1997. By that time there were some 130 plants of this clone growing on farm, all appearing healthy, but some scattered plants of *Giant Cavendish* on the same farm were seriously infected; so the farmer believed the clone he selected was resistant to Fusarium wilt. Resistance to Fusarium race 4 of this new clone, designated *GCTCV-218*, was confirmed following a series of tests conducted at TBRI experimental farm during 1998-1999.

In 2000, an extensive trial on *GCTCV-218* was conducted on 40 ha involving a total of 197 farmers in central and southern part of Taiwan and on a 15 ha corporate farm at TBRI. Results of this study revealed that both disease resistance and higher-yielding characteristics of this new clone were fairly stable across a wide range of environment regimes. Fusarium wilt incidence on *GCTCV-218* averaged 4.1%, ranging from 1.6 to 12.2%, which is significantly lower than 9.8% of the wilt-tolerant variety *Tai-Chiao No. 1* and 29.6% of the wilt-susceptible variety *Giant Cavendish*. The weight of bunches harvested from *GCCV-218* averaged 30.6 kg, which is 10.1 kg on average heavier than that of *Giant Cavendish*. The huge increased bunch weight is due to the increased number of hands, not to the size of hands. The last point is important because the hands if too large would be unacceptable to the market.

The new clone, about 2.8 m tall, also beats the *Giant Cavendish* in other agronomic traits such as having more robust pseudostem, stronger petioles, thicker leaves, better hand formation, and being more uniform in the size of hands. The fruit was of high quality and received positive feedbacks from both the Japanese and local market. A comparison of salient features between *GCTCV-218* and its parent variety *Giant Cavendish* is shown in Table 4.

Because of the advantages of the high level of resistance to Fusariurn wilt and extraordinary high yield, most farmers express great interest in planting this new clone. It is estimated that planting *GCTCV-218* to replace Giant Cavendish would result in an increased yield from 32.4 to 51.6 tons/ha on Fusarium-infested farms, and from 38.4 to 54.5 tons/ha on clean farms, accounting for 59% and 42% of yield increase, respectively. The clone, officially named Formosana, is scheduled for release for commercial planting, beginning January 2002. Mass propagation of tissue cultured plantlets of this new variety

is in progress, with a target of producing 2 million for distribution to farmers in the first year.

Table 4. Comparison of salient features between Formosana and Giant Cavendish on the first plant crop.

Variety	Plant height (cm)	Pseudostem girth (cm)	Leaf ratio	No. of hand/ bunch	No. of finger/ bunch	Bunch weight (kg)	Crop cycle (month)
GC	274	73	2.50	8.5	147	21.3	12
FM	281	82	2.33	11.5	191	30.2	13

GC: Giant Cavendish, FM: Formosana; Data are means of two crop cycles.

CONCLUSION

For decades, banana production in Taiwan has been greatly challenged by the intractable Fusarium wilt problem. For the control, the susceptible variety *Giant Cavendish* must be replaced by the resistant variety. For obtaining a resistant Cavendish whose breeding based on crossing has been seriously handicapped by the female seed sterility problem, an appropriate method for breeding must be developed. Because in vitro propagation of banana has shown the potential for producing substantial genetic variability, an innovative approach based on somaclonal variation was taken by TBRI in 1984. Much interest, support, and effort have been devoted in this direction over the past 16 years leading to the continued discovery of many interesting, useful resistant clones and to the release of two better resistant varieties, *Tai-Chiao No. 1* and *Tai-Chiao No. 3*, for commercial production. They are now planted in about 1,600 ha of infested farms, saving the banana industry from destruction by Fusarium wilt. Although these two resistant varieties enabled growers to resume banana production on many abandoned infested farms, moderate success has been obtained because both are only mediocre in the level of resistance and in productivity.

Of most significant achievement made by this breeding program was the development most recently of a new superb resistant variety *Formosana*. This new variety beats not only the above two resistant varieties but also the parent variety Giant Cavendish in terms of disease resistance and horticultural performance, a breakthrough in banana breeding. To my best knowledge, this is the most productive Cavendish not seen elsewhere. It is scheduled for release for commercial planting in 2002 for replacing *Tai-Chiao No. 1* and *Tai-Chiao No. 3*. Commercialization of *Formosana* would have great impact on banana production in Taiwan. First, it would reduce the loss of production to Fusarium wilt from the present 15% level to less than 5% every year. Second, the yield per hectare would be increased by over 50%, hence greatly reducing the production cost , one of the key constraints for banana production in Taiwan. Third, for decades, Taiwan banana has been suffering from a seasonal uneven ripening problem that greatly reduces its competitiveness in the Japanese market. Planting *Formosana* to replace Giant Cavendish will resolve this problem for bananas produced by *Formosana* ripen evenly. Fourth, by having a variety replacement program aiming at 1000 ha in 2002, it is expected that the

amount of exported banana would be increased from the present 2 million boxes per annum up to over 5 million in 2003. More importantly, it is believed that more planting of *Formosana* in future has the great potential for Taiwan bananas to enter the new export markets because of the lower production cost and better fruit quality, thus more competitive, of this new variety. In comparison with the traditional banana breeding based on crossing (pollinating) which has not produced any improved Cavendish variety for commercial use over the past 70 years, the somaclonal variation breeding has proved to be an efficient, useful approach for the improvement of Cavendish cultivars. The somaclonal variation approach has the following features, attributing to the success of this breeding program.

1. A wide range of genetic variability is found among in vitro mass-produced plants, offering a high probability of success in selecting desirable mutated traits'. As shown in this study, the chance of success of selection for resistance to Fusarium wilt was about two to three clones for every 10,000 plants screened(10).
2. The mutated traits occurring in somaclonal variation including resistance to Fusarium wilt are genetically stable, as demonstrated by the new variety *Tai-Chiao No. 1*, Since its selection in 1988 and commercialization in 1992, about 5 million tissue cultured plants of *Tai-Chiao No. 1* have been propagated and distributed to farmers. The agronomic traits and level of resistance have shown to be consistent over the years(10).
3. Although most resistant clones originally obtained from the breeding program have major deficiencies, somaclonal variation approach offers the chance to select the improved types from their *in vitro*-derived progenies(10). Thus, breeding of banana this way can be like for other crops, making it is much less a 'dead-end' game. The secondary semi-dwarf improved variant *TC1-229* and tertiary semi-dwarf improved variant *TC1-600* obtained from the taller parent *Tai-Chiao No. 1*.(10) are cases in point.
4. The tissue culture program, begun 1983, has produced more than 2 million seedlings each year for use by farmers. Work on selection, through linkage to this tissue culture program, also can be made on commercial farms in collaboration with farmers, thus broadening the genetic variability for selection and making the selection cheaper as well. The best resistant variety *Formosana* was discovered this way.

Banana breeding by somaclonal variation approach is now widely used in many other breeding programs in various countries. In addition to Cavendish, it is believed that this method would be applicable to other types of banana and for inducing resistance to others major diseases including black ,Sigatoka, nematode, and even to viral diseases as well.

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