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Transmission of banana bunchy top virus by aphids to banana plantlets from tissue culture

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Abstract. Among the three temperatures tested high temperature is more favorable for aphid transmission of banana bunchy top virus (BBTV) to banana plantlets than low temperature. When banana aphids (*Pentalonia nigronervosa* Cog.) on diseased plantlets were kept at 16°C for 1 month before being transferred to feed on healthy plantlets, the disease agent could not be transmitted. However, when the feeding of aphids on diseased plants was carried out at 16°C for only 2 or 24 h, all the inoculated plantlets developed bunchy top symptoms. Transmission rate of BBTV was directly correlated with the number of viruliferous aphids feeding on the plantlets and is inversely correlated with age of host plantlets. About 53% of single viruliferous aphids were able to transmit BBTV when 1-month-old plantlets were used. The incubation period of BBTV was inversely correlated with age of host plantlets. For 1-month-old plantlets from tissue culture, the shortest incubation period was 15 days. Experimental results suggest that using banana plantlets in transmission test is of great advantage. They are easy to be obtained in large quantity and require less space and time for experiments than seedlings from suckers.

Key words: Aphid transmission; Banana bunchy top virus; Banana plantlet; *Musa acuminata*; *Pentalonia nigronervosa*.

Introduction

Bunchy top is the most destructive virus disease of bananas in the Eastern Hemisphere (Stover, 1972). The disease was first reported from Fuji in 1889 and was found in Taiwan in 1900 (Sun, 1961).

Banana bunchy top virus (BBTV) has been considered to be a possible luteovirus by Matthews (1982) based on the disease characteristics of being transmitted in a persistent manner by aphids and causing phloem damage. Research progress on this disease has been very slow in comparison with other serious virus diseases because of inability to purify the virus despite numerous previous attempts (Dale *et al.*, 1986; Wu, 1987). The difficulty is mainly due to the presence of large amount of latex and phenolic compounds in banana plants which interfere with virus extraction and purification.

Recently purification of BBTV was finally achieved by pulverizing the disease banana tissues pre-frozen in liquid nitrogen to reduce the interference by latex and phenolic compounds, and by stirring and incubating of the viral extract at low temperature followed by low/high speed centrifugation to remove contaminants which prevented the detection of BBTV by electron microscope observation and UV scanning (Wu and Su, 1990a). BBTV was found to be a small luteovirus (20-22 nm in diameter) consisting of single-stranded RNA with a relative molecular mass (M_r) of 2.0×10^6 and coat protein subunit with a M_r of 21,000.

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Subsequently monoclonal antibodies against BBTV were obtained (Wu and Su, 1990b).

In recent years banana plantlets from tissue culture have become a popular planting material in Taiwan. (Su *et al.*, 1986). It was therefore, necessary to develop a serological test with monoclonal antibodies for detecting BBTV in the plantlets to assure that plantlets used for plantings are bunchy top-free (Wu and Su, 1990b). During this experiment, it was preliminarily found that the transmission of BBTV by aphids to banana plantlets was much more efficient and convenient than to banana seedlings from suckers used by Sun (1961) and Magee (1927; 1940). The study was consequently expanded, and results from this study are described in this paper.

Materials and Methods

Plantlets of Cavendish banana (*Musa acuminata* Colla) were obtained following the method described by Hwang *et al.* (1984). Banana aphids (*P. nigronervosa* Cog.) were raised on banana plantlets in screen cages.

For studying the effect of acquisition temperature on efficiency of transmission, aphids on diseased plantlets in the screen cage were kept in growth chambers at 16, 20 and 27°C for 1 month. Aphids were then transferred to feed on 1-month-old healthy plantlets (5 aphids/plantlet) for 2 days and killed by spraying with malathion at 1000 ppm. Inoculated plantlets were incubated at 30°C and the disease was recorded after 75 days.

To determine the effect of short-term exposure to low temperature on transmission of BBTV, aphids starved at 16°C for 1 h were transferred to feed for 2 or 24 h on diseased banana plantlets with the same pre-treatment and were then transferred to feed on 1-month-old healthy plantlets at 16°C. After feeding for 2 days, aphids were killed and inoculated plantlets were incubated at 30°C. The disease was recorded after 60 days. Four plantlets each with 30 aphids were used for each treatment.

The experiments concerning the relation between aphid number and transmission rate were carried out at room temperature. Aphids fed on diseased banana plantlets for 2 days to acquire BBTV were transferred to feed on healthy plantlets. One, 5 or 10 aphids for 1-month-old plantlets and 2, 15, 20 or 30 aphids for 4-month-old plantlets were used. After feeding for 2 days, aphids were killed and the disease was recorded after 75 days.

To study the effect of temperature on incubation period, each 2-month-old healthy banana plantlet was inoculated with BBTV by exposure to 30 viruliferous aphids for 2 days, and incubated in growth chambers at 16, 20 or 30°C for disease development. Those plantlets which did not develop disease symptoms at 16 and 20°C on 75 days were transferred to 30°C for further observation.

For studying the relation between plantlet age and incubation period, 1-month-old plantlets were exposed each to 5 aphids, while 2- and 6-month-old plantlets were exposed each to 15 aphids. After 2-day feeding, aphids were killed. Inoculated plantlets were incubated at 30°C and observed daily.

Results

Among the three temperatures used for keeping aphids on diseased plantlets, 27°C was the best for transmission of BBTV to healthy plantlets followed by 20°C (Table 1). When fed at 16°C for 1 month, the vectors could not efficiently transmit BBTV. However, when the feeding of aphids on diseased plants were carried out at 16°C for only 2 or 24 h, all the inoculated plantlets developed bunchy top symptoms.

Table 1. *Effects of acquisition temperature on efficiency of transmission of BBTV by aphids to 1-month-old banana plantlets (5 aphids for each plantlet)*

Acquisition temperature (°C)	No. of plantlets tested	Transmission rate (%)
16	14	0
20	20	55
27	17	100

When 1-month-old banana plantlets were used, about 53% of single aphids were able to transmit BBTV (Table 2). Transmission rate was 100% when 5 or more aphids were used to inoculate each plantlet. Transmission efficiency was greatly decreased when 4-month-old plantlets were used. With 2 aphids for each plantlet the transmission rate was only about 8%, while with 20 the transmission rate was about 86% (Table 2). The 100% transmission rate was achieved only when 30 aphids per plantlet was used.

Table 2. *Effects of aphid number on transmission of BBTV to 1- or 4-month-old banana plantlets in greenhouse*

Age of plantlet	No. of aphid per plantlet	No. of plantlets tested	Transmission rate (%)
1-month-old	1	15	53
	5	15	100
	10	15	100
4-month-old	2	12	8
	15	14	79
	20	7	86
	30	14	100

Among the three incubation temperatures tested, 30°C was the best for disease development with all the test plantlets (2-month-old) showing disease symptoms within the test periods (Table 3). The minimum and maximum periods required for appearance of disease symptoms at this temperature were 15 and 30 days, respectively, and the average incubation period was 25 days. At 20°C, only 1 out of 5 plantlets tested showed slight disease symptoms after 41 days. The other 4 plantlets remained healthy on 75 days. However, when they were transferred to 30°C, all of them showed disease symptoms within 3 weeks. None of the plantlets incubated at 16°C showed any disease symptoms on 75 days. Again all of them showed disease symptoms within 3 weeks after being transferred to 30°C.

Table 3. *Effects of incubation temperature on development of bunchy top symptoms on 2-month-old banana plantlets when they were inoculated each with 30 viruliferous aphids*

Incubation temperature (°C)	Disease incidence on 75 days	Final transmission rate[a]
16	0/5	5/5
20	1/5	5/5
30	7/7	7/7

[a]The rates were counted when all symptomless plantlets were further incubated in 30°C for 3 weeks.

The average incubation periods for plantlets at the age of 1, 2 and 6 months were 16, 25 and 37 days, respectively (Table 4). The ranges of incubation period for 1-, 2- and 6-month-old plantlets were 15-18, 15-30 and 31-42 days, respectively.

Table 4. *Effects of age of banana plantlets on incubation period of BBTV as viruliferous aphids were used to inoculate the plantlets*

Age of plantlet (month)	Average height of plantlet (cm)	No. of plantlet tested	Incubation period (day)		
			Min.	Max.	Average
1	10	9	15	18	16
2	15	6	15	30	25
6	35	6	31	42	37

Discussion

When aphids were fed on diseased banana plantlets at 16°C for 1 month before being transferred to feed on healthy plantlets, the disease agent could not be transmitted. The transmission rates were 55 and 100% when BBTV acquisition temperature was 20 and 27°C, respectively. Although aphid activity at 16 and 20°C was very low, inefficiency of BBTV transmission appeared to be due to low concentration of the virus in the host tissues resulting from low multiplication rate of BBTV at these temperatures because 100% transmission rate was obtained when aphids were fed on diseased plantlets at 16°C for only 2 h. This explanation is compatible with the finding that BBTV-infected banana plantlets did not develop symptoms at 16°C and showed only slight symptoms at 20°C. This is also compatible with the finding that the concentration of BBTV of banana leaves in winter is lower than that in spring and fall (Wu and Su, 1990a). Again this is consistent with the field observation that bunchy top disease incidence is higher in spring and fall than in winter, although the aphid population is highest in winter.

Results from this study showed that BBTV transmission rate is directly correlated with the number of viruliferous aphids feeding on the plantlets and is inversely correlated with age of host plantlets. The incubation period of BBTV was inversely correlated with age of host plantlets. It is possible that young plantlets may be more suitable for multiplication of BBTV, or that young plantlets require less BBTV for showing disease symptoms. By using 1-month-old banana plantlets, transmission of BBTV by single aphid was demonstrated for the first time in this study.

Plantlets from tissue culture have several advantages over seedlings from suckers for insect transmission studies. Diseased-free plantlets are easy to be

maintained and fast to be obtained in large quantity with uniform shape and same age for experiments. When compared with seedlings as used by Sun (1961) and Magee (1940), plantlets also occupy less space and require less time for symptom development.

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Literature Cited

Dale, J. L., D. A. Phillips, and J. N. Parry. 1986. Double-stranded RNA in banana plants with bunchy-top disease. J. Gen. Virol. **67:** 371-375.

Hwang, S. C., C. L. Chen, J. C. Lin, and H. L. Lin. 1984. Cultivation of banana using plantlets from meristem culture. Hort Science **19:** 231-233.

Magee, C. J. 1927, Investigation on the bunchy top disease of the banana. Bull Comm. Aust. Council Sci. Ind. Res. **30:** 1-64.

Magee, C. J. 1940. Transmission studies on the banana bunchy top virus. J. Aust. Inst. Agric. Sci. **6:** 109-110.

Matthews, R. E. F. 1982. Classification and nomenclature of viruses. Intervirology **17:** 1-200.

Stover, R. H. 1972. Banana, Plantain and Abaca Diseases. Commonw. Mycol. Inst., Kew, England.

Su, H. J., S. C. Hwang, and W. H. Ko. 1986. Fusarial wilt of Cavendish bananas in Taiwan. Plant Dis. **70:** 814-818.

Sun, S. K. 1961. Studies on the bunchy-top disease of banana. Spec. Publ. Coll. Agric. Taiwan Univ. **10:** 82-109.

Wu, R.Y. 1987. Characterization and Monoclonal Antibodies of the Virus Causing Banana Bunchy Top. Ph. D. Thesis, National Taiwan Univ., Taipei.

Wu, R. Y. and H. J. Su. 1990a. Purification and characterization of banana bunchy top virus. J. Phytopathol. (in press)

Wu, R. Y. and H. J. Su. 1990b. Production of monoclonal antibodies against banana bunchy top virus and their use in enzyme-linked immunosorbent assay. J. Phytopathol. (in press)

利用香蕉組織培養苗進行蕉蚜傳播香蕉萎縮病毒之研究

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利用蕉蚜對香蕉組織培養苗進行傳播實驗，在不同處理下發現27°C之獲毒溫度較20°C或16°C適合於病毒之傳播。在16°C中經過一個月後，病株即喪失傳病力，但若只經過2小時或1天，則不影響傳病力。在蚜蟲隻數方面則發現傳播率與隻數成正比。此一傳播率另與蕉苗年齡成反比。利用單隻蕉蚜發現已可傳病，且對一月大之小蕉苗其傳播率可達53%。至於發病潛伏期則大致與蕉苗年齡成正比，對於一月大之蕉苗，其潛伏期最短是15日。由以上之實驗顯示利用組織培養小蕉苗進行蚜蟲傳播實驗十分便利，因爲既可大量取得同齡材料，又比吸芽蕉苗可以節省空間及觀察發病之時間。