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SURVEY ON INCIDENCES AND SEVERITY OF VIRUS DISEASES OF BANANA IN SRI LANKA

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ABSTRACT

Banana is one of the major fruit crops grown in Sri Lanka. The extent of banana cultivation has been expanded during the past few years. Four virus diseases, BBrMV, BSV, BBTV and BMV cause considerable damage to the banana yield and fruit quality. Mix infections of two or more of these viruses cause severe reduction of bunch size. A survey was conducted to study the spread of virus diseases in various districts in Sri Lanka. ELISA and visual symptoms of four virus diseases were used as tools for disease assessment. According to the results of the survey, 82% and 59% of the *Embul* banana cultivation is infected with BBrMV and BSV respectively. Some symptomless *Embul* banana plants collected from Udapola and Mandaramnuwara areas were free from virus infections. High incidence of BBrMV could be observed in Ash plantain cultivation grown in Neelabamma area. *Aphis gossypii* collected from infected Ash plantain plants were tested using ELISA and BBrMV could be detected in their bodies. Banana mosaic disease incidences were low and detected in *Embul* banana cultivations in Anuradhapura, Kaluthara Kandy, Kegalle, Mathara and Matale districts. BBT has an island wide distribution. BBrMV could be detected in *Seenikesel, Anamalu, Ashplantain, Alumondan, Rathambala, Kolikuttu,* and *Ambon*. BSV infections were confirmed in *Anamalu, Rathambala, Ash plantain, Kolikuttu* and *Ambon*.

INTRODUCTION

Banana (*Musa* sp.) is one of the major fruit crops grown in Sri Lanka. The extent of banana cultivation is nearly 60,000 ha and the annual production is 780,000 t. Four virus diseases, banana bract mosaic virus (BBrMV), banana streak virus (BSV), banana bunchy top virus (BBTV) and banana mosaic virus (BMV) cause considerable damage to the fruit quality and the banana yield.

Banana streak virus was first isolated in 1985 (Lockhart, 1986). BSV belongs to the Badnavirus group and the virions are bacilliform in shape (120-150 x 30 nm.), containing a circular ds-DNA genome (Lockhart & Olszewski, 1993). BSV transmit by mealy bugs, *Planococcus citri* and *Saccharicoccus sacchari,* in semi persistent manner (Lockhart & Autrey 1988). David Jones reported BSV from Sri Lanka in 1995. Banana streak virus changes the normal appearance of the banana plant. A few banana leaves remain erect at the terminal part of the plant while older leaves are drooping. The yellow streaks appear parallel to the veins of the leaves, turn to brown and then to black. Internal necrosis can be observed and splits on the base of the leaves cause breaking of the leaves and drooping around the pseudostem. In the Ivory Coast, studies on AAA Cavendish subgroup cultivar Poyo showed yield losses over two cropping cycles of between 7% on plants with mild symptoms and 90% on plants with severe symptoms (Frison and Sharrock, 1998).

Banana bract mosaic virus belongs to the family Potyviridae, flexuous rod-shaped virus transmitted by aphid vectors-*Aphis gossypii*, *Pentalonia*

nigronervosa, *Rhopalosipium maidis*. In 1995, David Jones reported BBrMV in Sri Lanka. It induces red diamond shaped patches on the pseudostem and midrib of the leaves and dark patches on the bracts of the banana flower. Splitting of the base of the pseudostem is also visible. An infected plant produce distorted fruits and bunches.

Occurrence of BBTV in Colombo district was first reported in 1913 (Gadd, 1926; Small, 1928). This disease was a limiting factor for banana cultivations in Central and Western Provinces of the island. At that time, the disease has not been recorded in Tissa area but disease plants have been found in Hambantota district (Houston and Park, 1930). This virus belongs to the luteovirus group (Matthews, 1982). Houston and Park (1930) confirmed that the BBTV is transmitted by banana aphid (*Pentalonia nigronervosa*). They also found banana cultivar *Hondarawala* is less susceptible to the disease compared to the cultivar *Kolikuttu*. It causes stunting of the infected plant, reduces the normal distance between two leaves, which leads to gather leaves at the apical part of the plant. Infection of the virus causes reduction of leaf size and also causes marginal yellowing and necrosis of the leaves. When the plant is infected at the early stage of the growth, it is unable to produce a bunch, but infection during latter stage of the growth can produce small-distorted bunches with poor fruit quality.

Banana mosaic virus (cucumber mosaic virus serotype1) is a member of *Cucumovirus* group (28-30 nm diameter) with three positive sense RNA genome. It has been reported to have extensive host range with more than 800 plant species as hosts (Douine *et al.*, 1979). Over 60 species of aphid vectors transmit CMV in non-persistent manner (Francki *et al.*, 1979). This virus induces mosaic symptoms on leaves and if the temperature goes down it can cause heart rot and subsequent death of the plant.

With the expansion of the banana cultivations at commercial level, different varieties of banana suitable for different agro ecological conditions are grown under irrigated and rain fed conditions. But, these virus diseases have affected the banana production by reducing the quality and quantity of the yield. Hence the objectives of this study were to assess the spread of banana virus diseases in various districts of Sri Lanka and to estimate the severity of the four banana virus diseases. The local banana germplasm were evaluated for virus infections by visual symptoms and serological detection methods (Clark and Adams, 1977).

MATERIALS AND METHODS

Survey on virus diseases

The survey was carried out during 1996, 1997, 1998, 1999, 2000 and 2001 to assess the incidences of viral diseases of banana in various districts of Sri Lanka. Banana cultivations in different districts of Sri Lanka were evaluated for virus infections. Locations for the survey were selected after consulting the agriculture instructors of the Department of Agriculture in different district. From each district several locations were selected amounting to a total of 8-20 ha of banana plantations for this survey.

Preliminary diagnosis was based on the symptom expression. Disease assessments were based only on visual symptom expression and any symptom-less plant or latent infections were not included for the evaluation. ELISA confirmed presences of viruses in symptomatic plants. Samples were collected from individual field to confirm the virus infections and numbers of infected plants were counted to calculate the percentage of virus infections.

Samples were collected from symptom bearing plants as well as symptom-less plants for virus detection by ELISA. Collection of samples from individual banana plant was carried out from different parts of the plant: the base of the pseudostem, petioles of the leaves, base and the tip of the leaf lamina of young leaves and bracts of the flower. Collected samples were kept in labeled polythene bags in an icebox to avoid the exposure to high temperatures. Enzyme-linked Immunosorbent Assay (ELISA) was performed on collected banana samples to confirm the infections of BBrMV, BSV, BBTV, and CMV.

Aphid vectors were also collected from the pseudostem of some banana plants. Since they are vectors of some banana diseases, they were also tested to confirm the presence of acquired viruses. ELISA test was done to confirm the virus infections.

Enzyme-Linked Immunosorbent Assay (ELISA)

Laboratory virus confirmation tests were carried out at Virus Indexing Center, Horticultural Crop Research and development Institute, Gannoruwa. Double antibody sandwich ELISA (DAS- ELISA) was performed to confirm the virus infections of banana using commercial kits (Agdia 1000, Agdia Inc., Elkhart, IN, and USA For BBTV and BMV, Compound Direct ELISA procedure was used according to the Agdia protocol. Samples were collected from pseudostem, petioles, leaf lamina and bracts of each banana plant and pooled them to get one sample for testing.

ELISA plates were coated for BBrMV, BSV (polyclonal antibodies), BBTV and CMV serotype 1 by adding 100 µl of antibody solution into each ELISA well (prepared by dissolving antibody in coating buffer: 1.59 g of sodium carbonate, 2.93 g of sodium bicarbonate dissolved in 1000 ml of distilled water). Coated ELISA plates were incubated overnight at 4^0 C. Plant sap was extracted to confirm BBrMV and BSV from each 1 g sample in 1 ml of general sample extraction buffer (20.0 g of polyvinylpyrrolidone, MW 24 to 40,000, 1.3 g of sodium sulfite, 0.2 g of sodium azide, 2 g of powdered egg (chicken) albumin, Grade 11, 20.0 g of Tween-20 dissolved in 1000 ml. of phosphate-buffered saline Tween) using a leaf-roller tissue press. BBT extraction buffer (6.35 g of Tris-HCl, 1.18 g of Tris-base, 0.1% sodium N, N-Diethyldithocabamate, 5% sucrose dissolved in 1000 ml. of distilled water) and CMV extraction buffer (27.86 g of potassium hydrogen phosphate, 5.44 g of potassium dihydrogen phosphate dissolved in 1000 ml. of distilled water) was used to extract sap from each plant for BBT and CMV tests respectively. One hundred µl of extracted sap were placed in each well of the ELISA plate. Each plant sample was replicated three times in one ELISA plate. Positive and negative controls were also included in each ELISA plate. DAS-ELISA performed according to the Agdia protocol (Agdia Pathoscreen Kit, DAS ELISA, Alkaline phosphatase label, Agdia Incorporated, 30380 Country Road 6, Elkhart, Indiana 46514, USA) and at the end of the procedure, color reactions were recorded. ELISA plates were read with a Labsystem Microplate Photometer (model Multiscan EX) using a 405 nm filter. The threshold criterion used for determining a positive reaction was 2 x the average absorbance value of the healthy control.

RESULTS AND DISCUSSION

Results of the survey

Table 1 shows the percentage of BBrMV, BSV, BBTV and BMV infected *Embul* banana plants in different districts of the island. The lowest incidences of BBrMV and BSV were recorded in Nuwaraeliya district. *Ambon* is the popular variety among the farmers in the District and it did not show symptoms of BSV and BBrMV. But, low incidences of BBTV were detected. In Badulla district, *Embul* is not a popular variety and *Ambon* is the selection of most farmers.

Twenty-four ha *Embul* banana cultivations in Rideegama area of Kurunagala district showed high incidences of BBrMV. During 1999, 2000 and 2001, in Kegalle and Kandy districts, 57 ha of banana cultivations were surveyed for virus diseases. Virus-free *Embul* banana plants were observed and collected from Udapola area in the Kegalle district. In Matale district, 20 ha of banana cultivations were surveyed for virus diseases and heart-rot symptom were prominent in BMV infected plants.

Table 1. Percentage of virus infected *Embul* banana plants in different districts of Sri Lanka in 1999

District	*Percentage of Virus Infected Embul Banana Plants (%)*			
	BBrMV[1]	*BSV*[2]	*BBTV*[3]	*BMV*[4]
Anuradhapura	90	20	2	10
Badulla	98	1	2	0
Colombo	75	65	10	0
Gampaha	91	79	3	0
Hambanthota	98	85	2	0
Kaluthara	87	70	2	3
Kandy	95	60	4	2
Kegalle	70	60	3	2
Kurunagala	80	65	1	0
Matale	70	55	3	3
Mathara	99	90	1	1
Monaragala	70	60	4	0
Nuwaraeliya	1	0	0	0
Plonnaruwa	100	99	1	0
Puttlem	97	1	1	0
Rathnapura	85	80	3	0

1. Banana bract mosaic virus: symptoms - Diamond shaped red patches on pseudostem, leaf base & leaf midribs. Diamond shaped dark patches on bracts of the inflorescence and small bunches, Distorted small fruits.
2. Banana streak virus: symptoms - Leaf rolling, vain thickening, small bunches, distorted small fruits, splitting of pseudostem and leaf base.
3. Banana bunchy top virus: symptoms-Stunting of plants, yellow streaks, marginal necrosis, no fruit formation.
4. Banana mosaic virus: symptoms - Leaf rolling, vain thickening, and Heart rot.

During 1998 tissue cultured *Embul* banana showed high percentage of BSV symptoms and also symptoms of BBrMV, in Matara district. The farmers expanded their banana cultivations in paddy lands as well as in highland areas with the irrigation facilities provided by the Mahaweli projects. *Embul* was the popular variety among farmers and they used BBrMV and BSV infected planting materials for their new cultivations. Planting of diseased materials in the region increased the virus disease incidences.

Farmers in Puttalam district cultivated Ash plantain, but there were a few *Embul* banana cultivations. High percentage of BBrMV infected Ash plantains with severe symptoms was observed. Aphids were collected from BBrMV infected Ash-plantain plants were tested using ELISA to confirm the presence of BBrMV in their bodies. The detection of BBrMV in aphids suggest that they spread the disease in this area.

Embul variety showed high incidences of BSV and BBrMV in Polonnaruwa district. There were splits on the base of the leaves due to severe BSV infection. Therefore, it was observed breaking of the leaves at early stage of development. The reductions of bunch size and yields were observed even with better management practices. Virus infected plants produced bunches with small-distorted fruits.

Table 2. Assessment of virus infections based on symptom expression

District/Year	*Area*	*Banana Cultivar Surveyed*	*Disease Incidences*			
			BBrMV	*BSV*	*BMV*	*BBTV*
Anuradhapura 1999	Maha Illuppallama	Embul	++++	++	+	+
	Puliyankulama	Kolikuttu	-	-	-	-
	Rathmalgahawawa	Ash plantain	-	-	-	-
	Kahatagasdigiliya	Seeni	-	-	-	-
	Mahapathana	Wadanakesel	-	-	-	-
Badulla -1999	Rambukpotha	Ambon	-	-	-	-
	Hinnarangolla	Embul	+++	+	-	+
		Kolikuttu	-	-	-	-
		Seeni	-	-	-	+
		Anamalu	+	-	-	+
		Ash plantain	++	-	-	-
Gampaha-1999	Gampaha	Embul	++++	+++	-	+
Hambanthota-1999	Hambanthota Ambalanthota	Embul	++++	+++	-	+
Kaluthara-1999	Kaluthara	Embul	++++	+++	-	+
Kandy-1999, 2000, 2001	Peradeniya	Embul	+++	++	+	+
	Kadugannawa	Seeni	++	-	-	-
	Kundasala	Kolikuttu	-	-	-	-
	Katugastota	Ambon	-	-	-	+
Kegalle 1999,2000, 2001	Udapola	Embul	++++	+++	+	+
	Rambukkana	Seeni	+++	-	-	-
	Mawanella	Kolikuttu	-	-	-	-
		Ambon	-	-	-	+
Kurunagala 1999	Rideegama	Ambon	-	-	-	-
	Mawathagama	Embul	++++	++	-	+
	Malsiripura	Ashplantain	-	-	-	+
		Puwalu	-	-	-	-
		Kolikuttu	-	-	-	-
		Rathambala	++	-	-	-
Matale-1999, 2000	Palapathwela	Ambon	-	-	-	-
	Idangamuwa	Embul	++++	+++	+	+
	Yatawatta	Ashplantain	++	-	-	-
		Seeni	++	-	-	+
		Kolikuttu	++	-	-	-
		Suwandel	-	-	-	+
		Puwalu	-	-	-	-

Continuation of Table 2

District /Year	Area	Banana cultivar surveyed	Disease incidences			
			BBrMV	BSV	BMV	BBTV
Mathara-1998	Hakurugoda Yaya	Embul	++++	+++	+	+
	Mamadala	Seeni	-	-	-	-
	Waligatta	Kolikuttu	-	-	-	-
	Weerawila	Ashplantain	++++	-	-	+
	Allegala					
	Beralihela					
	Morakatiya					
	Kochchigallara					
Monaragala 1999	Monaragala	Embul	++++	++	-	+
Nuwaraeliya 2000	Nuwaraeliya	Ambon	-	-	-	+
	Mandaramnuwara	Embul	+	-	-	+
	Mathurata					
Plonnaruwa 2001	Bakamuna	Embul	++++	+++	-	-
	Aralaganwila					
Puttalam 2001	Neelabamma	Ashplantain	++++	-	-	+
	Chillaw	Embul	++++	+	-	+
		Ambon	-	-	-	-
		Alumondan	-	-	-	-
		Kolikuttu	-	-	-	-
Rathnapura 2000	Kuruwita	Embul	++++	+++	-	+
	Ellawala	Seeni	+++	-	-	-
	Karandana	Anamalu	++	-	-	+
		Ambon	-	-	-	-

++++ Highly infected, ++ to +++ Moderately infected, + Low infection, - No infection

Even though the percentages of BBT infected plants are low, they showed very severe symptoms compared to other virus diseases. BBT infected plants were severely stunted and clearly detectable in the field. Early infections prevented bunch formation and late infections produced small low quality bunches. Most farmers cut the infected plants at the base and do not remove the infected rhizome. Therefore, new plants from remaining infected rhizomes contained high titer of BBTV. There were neglected BBT infected plants in the reservations along the main roads and streams.

ELISA results

According to the ELISA, high virus titer of BBrMV was observed in bracts and midribs of the leaves. Observed high incidences of BBrMV in younger plants as compared to the mother plant of the same bush. Continuous cultivation of infected *Embul* variety gradually increased the disease severity

of the following generations. High virus titers was detected from younger plants of the buses compared to old plants.

The *Embul* variety was found to be highly susceptible to BSV infection and Ash plantain, *Kolikuttu* and *Ambon* were moderately susceptible to the disease. *Rathambala* showed low incidences of BSV and it was not detected in *Seeni kesel, Puwalu*, and *Alumondan*. Virus-free varieties may be resistant to the BSV infection or escapes of the infection (Figure 1).

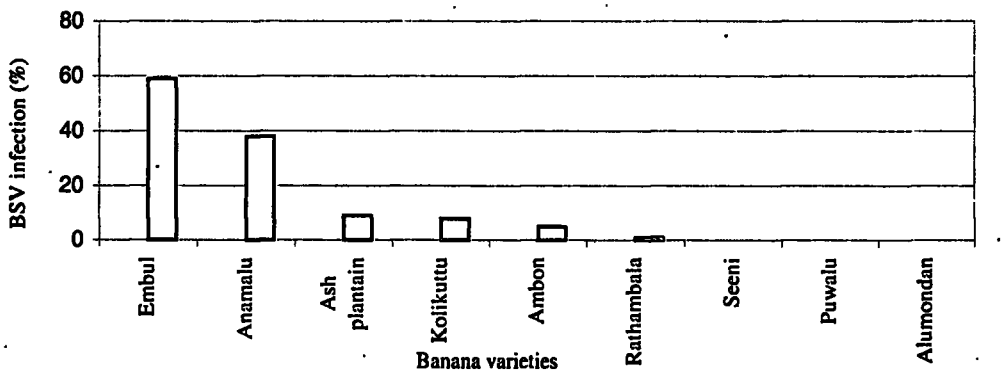

Figure 1. Percentage BSV infection of banana cultivars grown in different districts of Sri Lanka in 1999

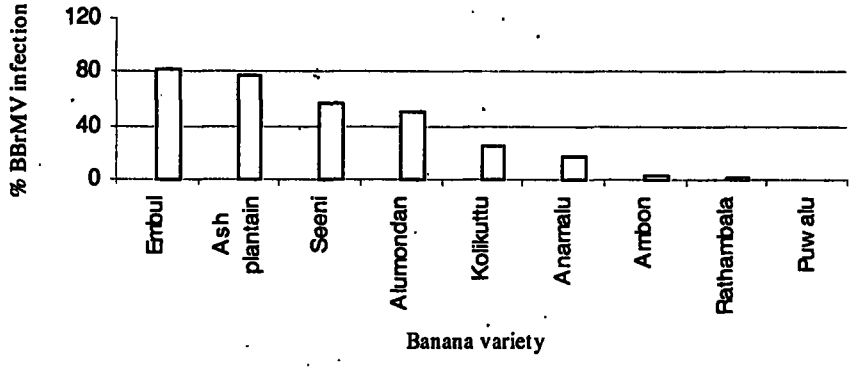

Figure 2. Percentage of BBrMV infection of banana cultivars grown in different districts of Sri Lanka in 1999

Embul and Ash plantain had the high percentage of BBrMV infection and they were highly susceptible to the disease (Figure 2). *Seeni kesel* and *Alumondan* showed susceptible reaction to BBrMV infection. Moderately susceptible reaction was observed in *Kolikuttu* and *Anamalu*. Banana varieties *Ambon* and *Rathambala* showed low incidences of BBrMV. *Puwalu* was free from BBrMV infection.

In Matara, Polonnaruwa, Hambantota, Puttalam and Anuradhapura districts, BBrMV incidences were found to be high (Figure 3). Propagation of BBrMV infected planting materials; stress due to climatic factors may be the

reasons for severe symptom expression. Lowest disease incidences were observed in Badulla and Nuwara Eliya Cultivation of BBrMV-free *Ambon* variety and cool climatic condition may be the reasons for low disease levels.

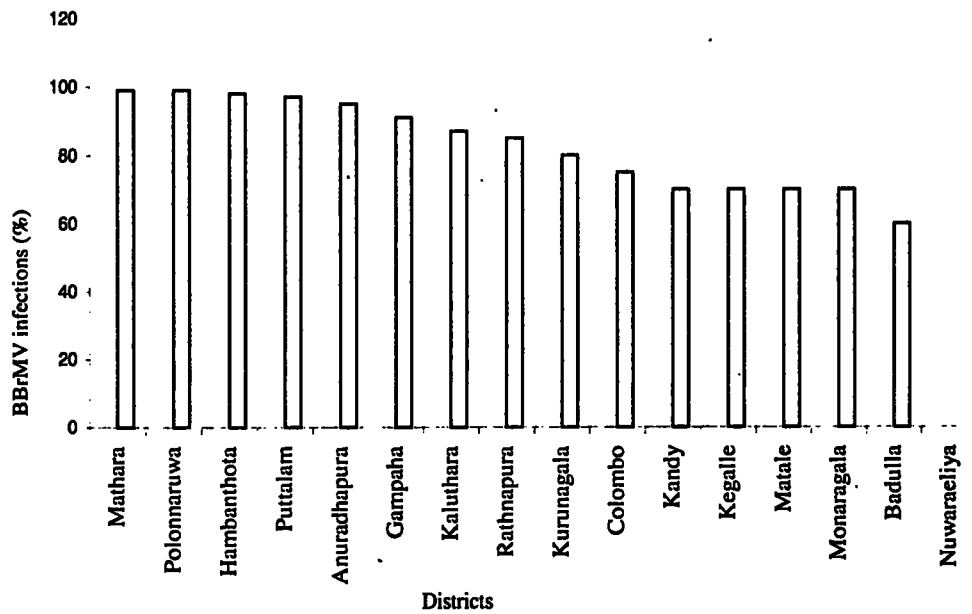

Figure 3. Percentage of BBrMV infection detected in banana varieties cultivated in different districts of Sri Lanka - Average of all varieties

The highest BSV incidences were recorded from Polonnaruwa district because of the extensive cultivation of susceptible *Embul* variety (Figure 4). Low BSV incidences were recorded from Nuwara Eliya and Badulla districts. This may be due to cultivation of BSV free *Ambon*. Ash plantain is the popular variety in Puttalam district where high incidences of BSV were detected.

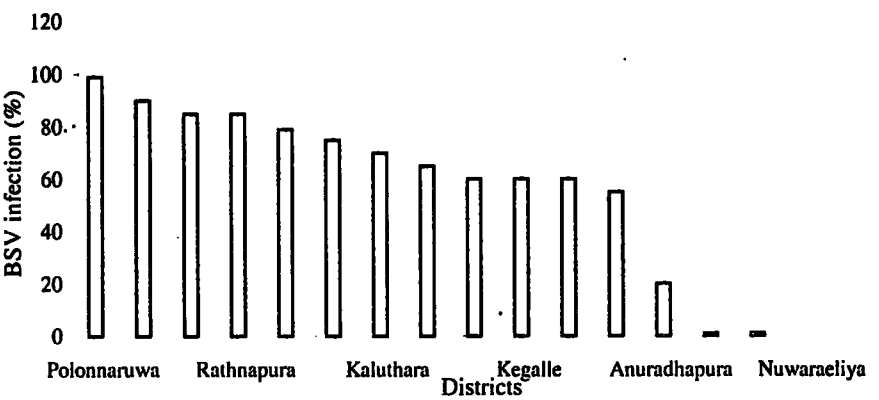

Figure 4. Percentage of BSV infection detected in banana varieties cultivated in different districts of Sri Lanka - Average of all varieties

BBrMV and BSV incidences declined with increasing elevation. The disease incidences were high in the areas with high temperature and sunny conditions. This may be due to the expression of some virus diseases under stress conditions. There were high disease incidences of BBrMV in districts where *Embul*, Ash plantain and *Seeni* varieties are grown. BBrMV and BSV spread mainly by infected planting material distributed from severely affected areas. Therefore, expansion of banana cultivations should be done with a great care. Eradication of virus infected plants and use of healthy plants for new cultivations will help to reduce the incidences, severity of banana diseases and promote the yield as well as the quality of the yield. Continuous cultivation of infected plants and mix infection of two or more viruses cause severe reduction of banana yields.

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