Jurnal Perlindungan Tanaman Indonesia, Vol. 22, No. 1, 2018: 1–12
DOI: 10.22146/jpti.23763

Research Article

First Report of Necrotic Spot Disease Caused by *Cactus virus X* on Dragon Fruit (*Hylocereus* spp.) in Peninsular Malaysia

Laporan Pertama Penyakit Bercak Nekrotik yang Disebabkan oleh Cactus virus X *pada Buah Naga (*Hylocereus *spp.) di Semenanjung Malaysia*

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Submitted April 6, 2017; accepted November 6, 2017

ABSTRACT

This study was conducted to detect the status of virus causing necrotic spot disease on dragon fruit and to recognize its geographical distribution in Peninsular Malaysia. Fifty posts of crops were randomly sampled from dragon fruit orchards. The symptoms were characterized and the pathogen was observed under transmission electron microscopy (TEM). Disease incidence and severity were plotted, while the disease occurrence was statistically analyzed under Duncan's Multiple Range Test (DMRT) of General Linear Modeling (GLM) procedure and Pearson correlation test. The disease was initiated by necrotic small mottle or spot on young shoot which then turned to orange. Those symptoms were also found on mature stems and fruits. They could quickly expand on unexposed symptomatic parts in orchards. The maximum incidence and severity of disease were recorded in Durian Tunggal (Malacca), i.e. 98 and 52%, respectively. TEM technique viewed the spindle-shaped inclusion bodies of virus within symptomatic stems at 4000 and 20,000× magnification which were suspected as *Cactus virus X* (CVX), a potexvirus with filamentous and rod shape and 515−520 nm in size. Statistically, disease occurrence in Malacca was significantly higher than those in other states, while the minimum was found in Terengganu. The strong regression of disease incidence and severity was shown by R value= 0.9484. There were no significant correlations of disease occurrence either with weather or cultural factors. Further study on the transmission of virus within the crops in the field is important to be carried out in order to monitor the spread of disease and to develop the integrated disease management strategies on dragon fruit in Peninsular Malaysia.

Keywords: necrotic spot, CVX, dragon fruit

INTISARI

Kajian ini dilakukan untuk mendeteksi status virus yang menyebabkan penyakit bercak nekrotik pada buah naga dan untuk mengetahui distribusi geografisnya di Semenanjung Malaysia. Lima puluh tiang tanaman disampel secara acak dari kebun buah naga. Gejala-gejala dikarakterisasi dan patogen diamati di bawah transmission electron microscopy *(TEM). Insidensi dan keparahan penyakit diplot, sedangkan kejadian penyakit secara statistik dianalisis menggunakan* Duncan Multiple Range Test (DMRT), General Linear Modeling (GLM) *dan uji* Pearson correlation*. Penyakit dimulai dengan bercak nekrotik kecil pada tunas muda dan kemudian berubah menjadi jingga. Gejala-gejala tersebut juga dijumpai pada batang dewasa dan buah. Penyakit dapat berkembang dengan cepat pada bagian bergejala yang tidak terlihat di kebun. Insidensi dan keparahan penyakit yang paling banyak ditemukan di Durian Tunggal (Melaka), yaitu masing-masing 98 dan 52%. Teknik TEM menunjukkan badan inklusi berbentuk benang dari virus pada batang yang bergejala pada perbesaran 4000 dan 20.000×yang diduga sebagai* Cactus virus X *(CVX), suatu potexvirus yang berbentuk benang dan batang serta berukuran panjang 515–520 nm. Secara statistik, kejadian penyakit di Melaka secara nyata lebih tinggi daripada di negara bagian lainnya, sedangkan kejadian penyakit yang paling sedikit dijumpai di Terengganu. Regresi insidensi dan keparahan penyakit yang kuat ditunjukkan oleh nilai R= 0,9484. Tidak ada korelasi nyata kejadian penyakit, baik dengan*

faktor cuaca maupun budidaya. Kajian lebih lanjut terkait penularan virus dalam tanaman di lapangan penting untuk dilakukan dalam rangka memantau penyebaran penyakit dan untuk mengembangkan strategi pengendalian penyakit yang terpadu pada buah naga di Semenanjung Malaysia.

Kata kunci: bercak nekrotik, buah naga, CVX

INTRODUCTION

Dragon fruit (*Hylocereus* spp.) is a climbing cacti species bearing the fruit characterized by dragon-like scales covering its skin (Mizrahi *et al.*, 2002). It is popular fruit in South America and has been consumed by the general population since the pre-Columbian times (Crane & Balerdi, 2005). French brought it into Vietnam about 100 years ago as ornamental crop for the king (Luders & McMahon, 2006).

The commercial cultivation of dragon fruit in Malaysia was initiated in Kluang (Johore), Kuala Pilah (Negeri Sembilan) and Sitiawan (Perak) at the beginning of 1999. However, the Golden Hope Company, which is located at Sungai Wangi Estate (Perak), had introduced and cultivated this crop in a large scale plantation two decades ago (Halimi & Satar, 2007). According to the statistics reported on 2006, Malaysia planted around 962.3 ha of dragon fruit with the total production about 2,534.2 ton (production value around US$3.5 millions) (Cheah & Zulkarnain, 2008).

Most of diseases on dragon fruit documented either in Malaysia or other countries were dominantly caused by plant pathogenic bacteria and fungi (Valencia-Botin *et al.*, 2003; Wang and Lin, 2005; Le Bellec *et al.*, 2006; Taba *et al.*, 2006; Palmateer *et al.*, 2007; Paull, 2007; Taba *et al.*, 2007; Hoa, 2008; Masratul-Hawa *et al.*, 2008; Masyahit *et al.*, 2008, 2009a, 2009b). Meanwhile, plant pathogenic virus, primarily *Cactus virus X* (CVX) has been also detected in California (Fudl-Allah *et al.*, 1983), Taiwan (Liou *et al.*, 2001; Liao *et al.*, 2003), and Japan (Keiko & Miki, 2001). Furthermore, the characteristic of this virus has been reported by Attanthom *et al.* (1978) and Liou *et al.* (2004).

However, there is no scientific report regarding on the occurrence and distribution of this pathogen in Peninsular Malaysia. Therefore, this study was carried out to detect the presence of plant pathogenic virus causing disease on dragon fruit and to recognize its geographical distribution on dragon fruit-growing areas in Peninsular Malaysia.

MATERIALS AND METHODS

Surveys of Dragon Fruit-Growing Areas

Surveys were conducted in dragon fruit-growing areas in Peninsular Malaysia since December 2007 until August 2008. At least two dragon fruit orchards from each sampling state were surveyed. Data of disease occurrence and cultural (age of crops and acreage of surveyed farms) were recorded. Longitude and latitude data were documented using GPS device (SILVA Multinav-Version 2.01) and then mapped with Mapinfo Software (Troy, New York; USA). Weather data i.e., relative humidity (RH), temperature and wind velocity were obtained from Malaysian Meteorological Department (period of 1998–2008).

Assessment of Disease

Fifty posts of dragon fruit plants were sampled using pseudo-random method with specifying an approximate W-shaped path from each surveyed plantation with at least about 0.4 ha in acreage. Each plot was generally occupied by 4 cuttings. Disease incidence (DI) was calculated by the following equation (Cooke, 2006):

$$DI = \frac{Number\ of\ diseased\ plant\ units}{Total\ number\ of\ plant\ units\ assessed} \times 100\%$$

Meanwhile, disease severity (DS) was determined according to alternative rating scale proposed by Bowen (2004) in which scale 0 = no symptom, scale 1 = 0–20% of severity level on infected plants, scale 2 = 20–40%, scale 3 = 40–60%, scale 4 = 60–80%, and scale 5 = 80–100%, respectively and then measured using the equation proposed by Kranz (1988) as follows:

$$DS = \frac{\sum(a \times b)}{N.Z} \times 100\%$$

N = Total number of sampled plant
Z = Highest score scale

In the meantime, the frequency of certain disease which occurred in surveyed area was computed using this following equation (Cooke, 2006):

$$Disease\ frequency = \frac{Infected\ area\ by\ certain\ diseased}{Total\ surveyed\ area} \times 100\%$$

Detection of Plant Pathogenic Virus

Suspected pathogenic virus was characterized by observing size and shape under Transmission Electron Microscopy (TEM) (LEO912AB, EFTEM) following procedure provided by Institute of Biological Science, University Putra Malaysia. The tissues were cut into 1 mm^3 slices and then put into fixative solution (4% of Glutaraldehyde) for 48 h at 4°C. Afterwards, the slices were washed with 0.1 M Sodium Cacodylate Buffer for three times in 30 min each and post-fixed with 1% Osmium Tetroxide solution for 2 h at 4°C. Again, the slices were washed with 0.1 M Sodium Cacodylate Buffer for three times in 30 min each. They were consecutively involved in serial dehydration within 35, 50, 75 and 95% acetone for 60 min each, as well as in 100% acetone during 120 min for three times.

Hereinafter, the slices were infiltrated into mixture of acetone and resin with the ratio of 1:1 and 1:3 for one day and one night, respectively, and 100% resin overnight and 2 h, consecutively. The samples were then placed into resin-filled up beam capsules and polymerized in oven at 60°C for 24–48 h.

Thick sectioning procedures were started with the cutting of samples into 1 μm sections using glass knife and ultramicrotome. They were then placed onto glass slide and stained with toluidine blue; the slides were dried on hot plate, while the stain was washed; the slides were observed under light microscope.

Meanwhile thin sectioning was initiated by the selection of interested area on samples. They were then cut for ultrathin section; the silver or golden sections were selected and picked up with a grid and dried using filter paper. The sections were consecutively stained and washed with uranyl acetate for 10 min and filtered alcohol, followed by lead-staining for 10 min and rinsing with double-distilled water. Finally, the samples were viewed under TEM.

Statistical Analysis

Test of normality for disease occurrence data (DI and DS) was previously employed (Cardoso *et al.*, 2004). The polynomial regression was analyzed using Microsoft Excel 2003 program (Microsoft Corporation, Washington; USA). Both data of disease occurrence under General Linear Modeling (GLM) procedure with Duncan's Multiple Range Test (DMRT) and Pearson correlation were analyzed using SAS® System for Windows V8 software (SAS Institute Cary, North California; USA).

RESULTS AND DISCUSSION

Cactus virus X (CVX) is considered as one of important plant pathologic virus on cacti species (Zimmermann & Granata, 2002). Early detection of CVX on dragon fruit (*Hylocereus* spp.) in Peninsular Malaysia could be the basic information in developing the appropriate strategies to prevent the spread of disease, particularly in investigating the virus transmission within crops in the field and in selecting the resistant dragon fruit varieties.

Surveys of Dragon Fruit-Growing Areas

The present study recorded that the red-fleshed species was planted nationwide in Peninsular Malaysia, followed by white-fleshed and yellow species, with age of crops 0.25−6 years and 0.4−10.11 ha in acreage and the altitude of 3.35−146.91 m above sea level (asl) (Table 1). The 43 surveyed orchards and the distribution of disease could be mapped as shown by Figure 1. The disease was widely distributed in Peninsular Malaysia and could quickly expand on unexposed infected plant parts in dragon fruit orchards. The 11-year of average weather data in Peninsular Malaysia affecting the establishment of current surveyed disease occurrences was also given in Figure 2.

Assessment of Disease

This disease initially appeared as necrotic small mottle or spot on base of young shoots and unripe fruits which later turned to orange. The secondary found pathogens such as *Alternaria* sp., *Botryosphaeria* sp., *Colletotrichum gloeosporioides*, *Curvularia* sp., *Fusarium* sp., *Monilinia* sp., *Pestalotiopsis* sp., *Phytophtora* sp., and *Thielaviopsis* sp. might cause the rot of infected tissues. Those symptoms were also found on some mature stems and ripe fruits (Figure 3). The highest disease occurrence was recorded in Durian Tunggal (Malacca), with DI and DS about 98 and 52%, respectively (Table 1). It was presupposed that the most severe disease in this orchard likely due to unwell-sanitized farm. The farmer did not prune the damaged stems and ignored debris of diseased stems around the farm (Figure 4).

The lowest disease prevalence was found in Mata Ayer (Kedah), while there were some zero incidences of disease in certain surveyed areas (Table 1). We found that the location of those areas were far away from infected orchards. Several farms were possibly not contaminated by this disease as they were well-sanitized by pruning, burning and then burying the in-

Figure 1. Distribution of necrotic spot disease on dragon fruit in Peninsular Malaysia

Table 1. The occurrence of necrotic spot disease on surveyed dragon-growing areas in Peninsular Malaysia

Location[a]	Dragon fruit species	Farm environment	Altitude (m asl)	Age (years)[b]	Acreage (ha)[c]	Occurrence (%)	
						DI	DS
Johor							
Batu Pahat	Rf (Hp)	Rubber plantation	12.50	4.5	1.42	0	0
Kluang	Rf (Hp)	Rubber plantation	81.38	4	1.42	70	23.2
Mersing	Rf (Hp), Wf (Hu), Yf (Sm)	Opened high hills	47.55	6	10.11	72	17.6
				Mean of disease occurrence[d]		47.33ab	13.60ab
				Disease frequency		66.67%	
Malacca							
Durian Tunggal	Rf (Hp)	Rubber plantation	10.36	6	3.24	98	52
Machap Umboo	Rf (Hp)	Rubber plantation	61.26	5	2.43	56	18.8
Naning	Rf (Hp)	Rubber plantation	42.36	1.5	1.21	62	19.2
				Mean of disease occurrence		72.00a	30.00a
				Disease frequency		100%	
Negeri Sembilan							
Rembau	Rf (Hp)	Village situtation	54.56	1.5	0.48	4	0.8
Kuala Pilah	Rf (Hp)	Rubber plantation	80.77	1.5	2.83	92	22.0
Pajam	Rf (Hp)	Rubber plantation	60.96	2.5	2.43	50	15.6
				Mean of disease occurrence		48.67ab	12.80ab
				Disease frequency		100%	
Selangor							
Sepang	Rf (Hp)	Village situation	51.20	3	0.65	0	0
Hulu Langat	Rf (Hp)	Rubber plantation	75.90	0.67	2.43	46	14.8
Sabak Bernam	Rf (Hp)	Village situation	13.41	3	1.21	62	17.6
				Mean of disease occurrence		36.00ab	10.80ab
				Disease frequency		66.67%	
Perak							
Slim River	Rf (Hp)	Rubber plantation	44.50	4	0.81	38	10.4
Teluk Intan	Rf (Hp)	Village situation	5.80	4	0.40	48	12.8
Tapah	Rf (Hp)	Rubber plantation	40.54	3.5	0.81	48	12.4
Batu Gajah	Rf (Hp)	Rubber plantation	42.36	2.5	0.61	48	14.8
Pantai Remis	Rf (Hp)	Intercropping	6.40	1.5	2.43	38	9.6
Taiping	Rf (Hp)	Intercropping	20.42	1	0.81	42	10.4
				Mean of disease occurrence		43.67ab	11.73ab
				Disease frequency		100%	

Table 1. (continued)

Location[a]	Dragon fruit species	Farm environment	Altitude (m asl)	Age (years)[b]	Acreage (ha)[c]	Occurrence (%)	
						DI	DS
Pahang							
Pekan	Rf (Hp)	Village forest	11.88	3	4.45	78	30
Kuantan	Rf (Hp)	Urban situation	17.37	4	2.02	24	7.2
Raub	Rf (Hp)	Rubber plantation	146.91	4	3.64	14	4
Jerantut	Rf (Hp)	Rubber plantation	121.31	0.5	1.62	0	0
				Mean of disease occurrence		29.00ab	10.30ab
				Disease frequency		75%	
Terengganu							
Paka	Rf (Hp)	Intercropping	8.53	0.6	0.48	54	15.6
Merchang	Rf (Hp)	Village situation	6.09	3	1.01	0	0
Marang	Rf (Hp)	Intercropping	4.57	2.5	2.02	0	0
Setiu	Rf (Hp)	Intercropping	5.79	1.5	0.40	14	4.4
Kerteh	Rf (Hp)	Village situation	11.27	2.5	0.81	0	0
				Mean of disease occurrence		13.60b	4.00b
				Disease frequency		40%	
Kelantan							
Batang Merbau	Rf (Hp) and Wf (Hu)	Experimental plot	51.81	3	0.40	0	0
Kota Bharu	Rf (Hp)	Experimental plot	10.97	2	0.40	10	2.4
Gua Musang	Rf (Hp)	Rubber plantation	31.67	4	1.21	0	0
Kuala Krai	Rf (Hp)	Village forest	34.74	2	2.02	54	16
				Mean of disease occurrence		16.00b	4.60b
				Disease frequency		50%	
Pulau Pinang							
Bukit Mertajam	Rf (Hp)	Village situation	30.78	3	2.02	54	15.2
Seberang Perai Tengah	Rf (Hp)	Village situation	22.55	1.5	0.81	36	9.6
Seberang Perai Utara	Rf (Hp)	Intercropping	9.44	5	5.95	40	13.2
				Mean of disease occurrence		43.33ab	12.67ab
				Disease frequency		100%	

Table 1. (continued)

Location[a]	Dragon fruit species	Farm environment	Altitude (m asl)	Age (years)[b]	Acreage (ha)[c]	Occurrence (%)	
						DI	DS
Kedah							
Merbau Pulas	Rf (Hp)	Village situation	14.63	0.58	0.40	0	0
Pokok Sena	Rf (Hp) and Yf (Sm)	Rubber plantation	19.81	0.4	0.40	0	0
Gurun	Rf (Hp)	Intercropping	25.60	2	2.43	0	0
Yan	Rf (Hp)	Intercropping	3.96	2	0.40	14	3.6
Ayer Hitam	Rf (Hp)	Rice field	3.35	2	0.81	78	36
Mata Ayer	Rf (Hp)	Rubber plantation	28.95	3.5	2.02	2	0.4
Pantai Kok	Rf (Hp)	Village situation	27.43	1.5	0.40	62	27.2
Ayer Hangat	Rf (Hp) and Wf (Hu)	Experimental plot	3.96	4	0.81	54	16
				Mean of disease occurrence		26.25ab	10.40ab
				Disease frequency		62.5%	
Perlis							
Beseri	Rf (Hp)	Intercropping	25.60	2	0.61	36	10.4
				Mean of disease occurrence		36.00ab	10.40ab
				Disease frequency		100%	

Remark: [a]Locations were arranged successively from the northern (Johor, Malacca and Negeri Sembilan), western (Selangor and Perak), eastern (Pahang, Terengganu and Kelantan) and southern (Pulau Pinang, Kedah and Perlis).

[b]Age of crops which were recorded until surveyed date.

[c]Acreage of cultivated orchards in acre. One acre, which equals to 0.4 ha, consists of approximately 449 posts with 4 plants per post.

[d]The number followed by same letter in one column indicated the insignificant different under DMRT at $\alpha = 5\%$.

Rf = red-fleshed species (Hp = *Hylocereus polyrhizus*); Wf = white-fleshed species (Hu = *Hylocereus undatus*); Yf = yellow-fleshed species (Sm = *Selenicereus megalanthus*); DI = dis ease incidence; DS = disease severity.

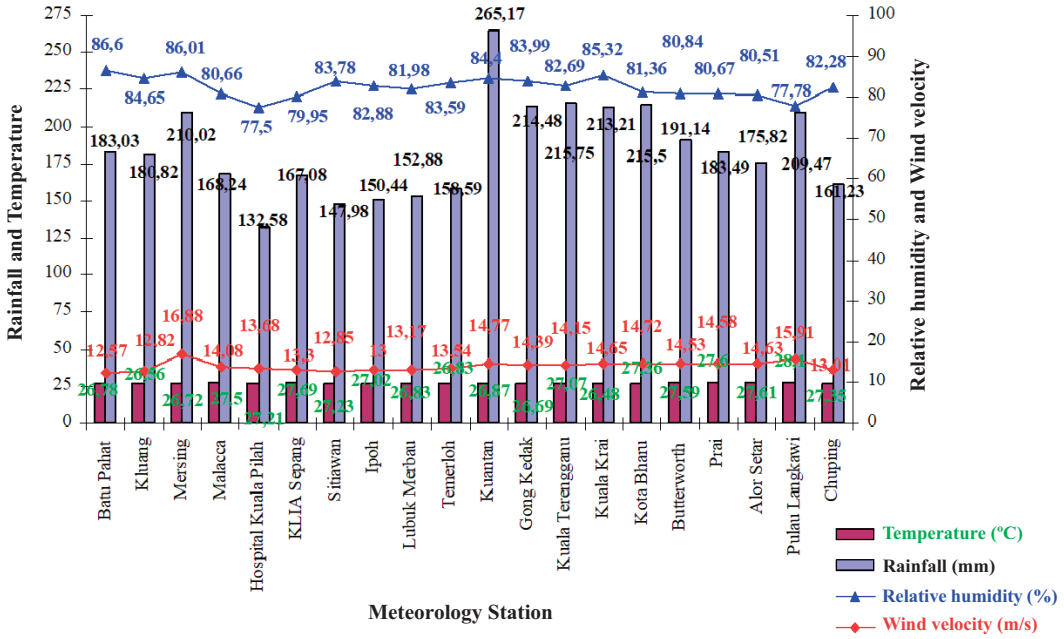

Figure 2. The 11-year (1998−2008) of average weather data in Peninsular Malaysia from Meteorological Malaysia Department

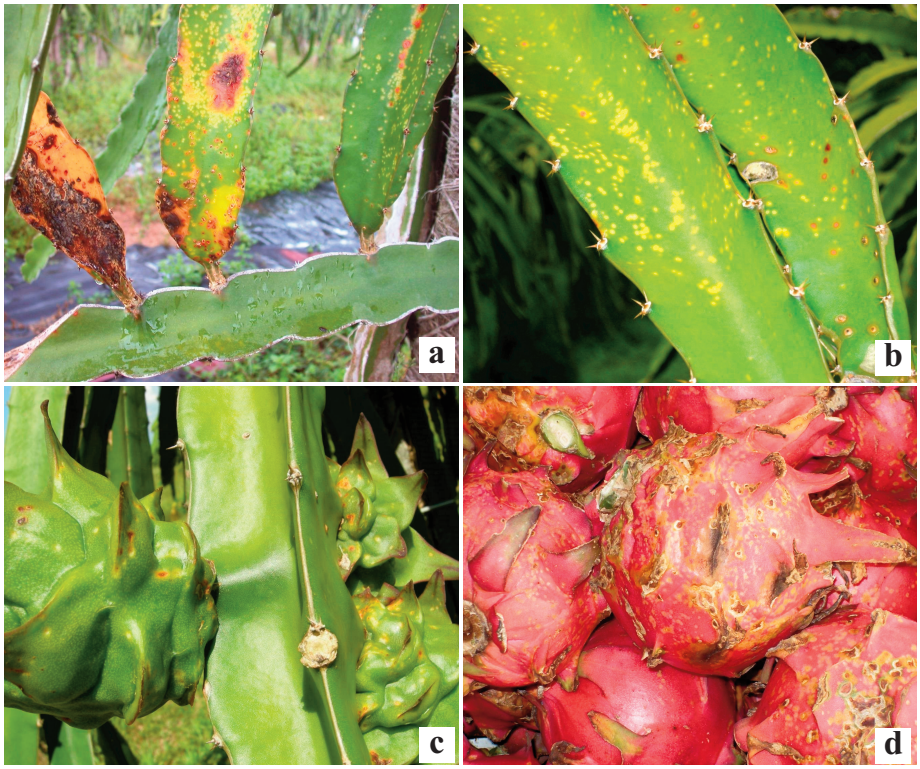

Figure 3. Symptoms of necrotic spot disease on infected young shoot (a), mature stem (b) and unripe (c) and ripe fruit (d) of dragon fruit

Figure 4. The unwell-sanitized farm in Durian Tunggal, Malacca

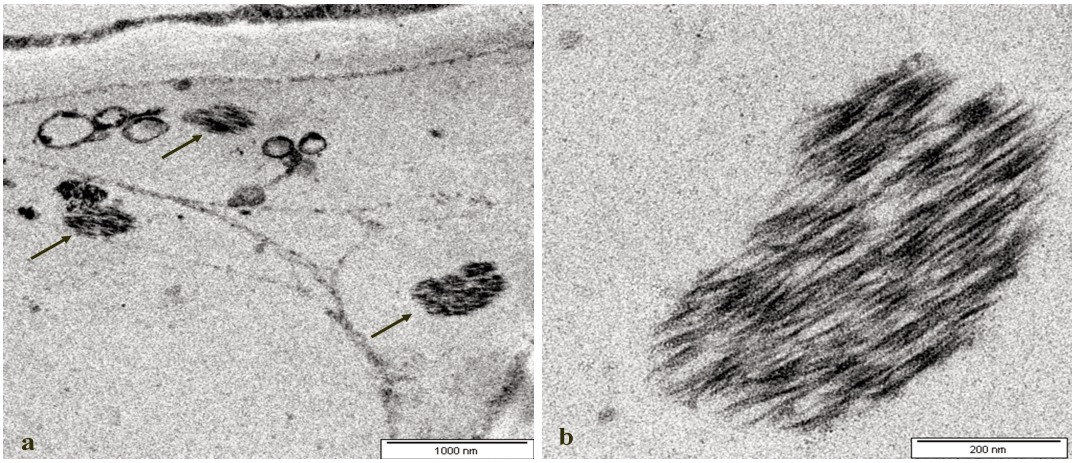

Figure 5. The spindle-shaped of viral inclusion bodies (arrow) on infected necrotic spot stems of dragon fruit under TEM at (a) 4000X and (b) 20,000X

fected plants out of the orchards. Le Bellec *et al.* (2006) recommended pruning all the injured and entangled stems at the first year after planting. Some orchards were regularly treated with effective microbe and application of insecticide, such as in Sepang (Selangor) and Jerantut (Pahang), respectively.

Moreover, this disease was encountered in all sampling states with the range of disease frequency around 40−100% (Table 1). This indicated that necrotic spot disease has infected at least one dragon fruit orchard in each surveyed state.

Detection of Plant Pathogenic Virus

Viral infection was detected on young stem with as necrotic small mottle or spot symptoms. TEM technique viewed the spindle-shaped inclusion bodies of suspected virus on infected stems at 4000 and 20,000X magnification (Figure 5). The inclusion bodies with similar shape have been observed in infected barrel cactus [*Ferrocactus acanthodes* (Lemaire) Britton & Rose] (Attanthom *et al.*, 1978) and white-fleshed species of dragon fruit (Fudl-Allah *et al.*, 1983) in California (USA).

Such inclusion bodies were suspected as aggregation of *Cactus virus X*, a filamentous rod potexvirus with 515−520 nm in size, which correspondingly caused distorted areoles, malformed spines, necrotic (Attanthom *et al.*, 1978) stunted and systemic mottle symptoms (Fudl-Allah *et al.*, 1983). That virus was also reported infecting same species in Taiwan with such symptoms on the infected white-fleshed dragon fruit (Liou *et al.*, 2001; Liao *et al.*, 2003) and on both white-fleshed and yellow species of dragon fruit in Okinawa Prefecture, Japan (Keiko & Miki, 2001). Its complete nucleotide sequence and genome organization have been determined as well (Liou *et al.*, 2004). Attanthom *et al.* (1978) characterized that this virus was transmissible by plant sap inoculation and grafting.

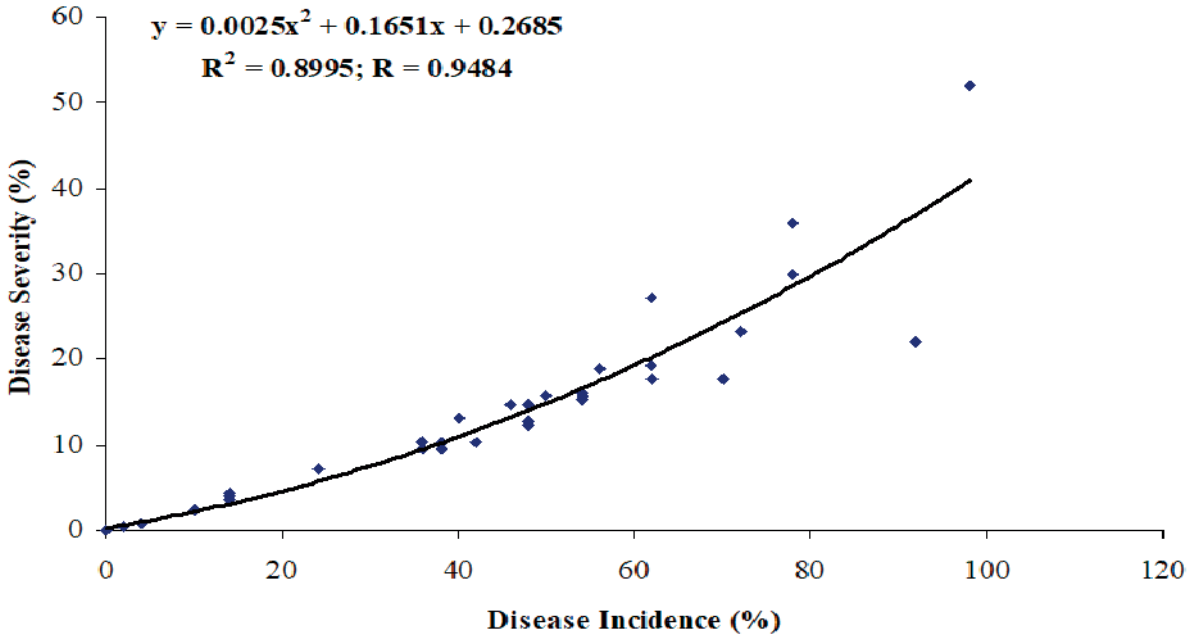

Figure 6. Disease incidence (DI) and disease severity (DS) relationships for necrotic spot disease

Table 2. The Pearson coefficient correlation (r value) of disease occurrence with weather and cultural factors

Disease occurrence	Temperature	Rainfall	RH	Wind velocity	Acreage of orchard	Age of crops	Altitude
Incidence	0.126	-0.098	-0.174	0.172	0.401	0.398	0.115
Severity	0.217	-0.018	-0.227	0.166	0.284	0.389	0.076

Statistical Analysis

The relationship of DI and DS for this surveyed disease was indicated by value of $R^2 = 0.8995$ with coefficient correlation, R = 0.9484 (Figure 6). This value clarified that the incidence of disease could strongly affect the increase of disease severity level. Seem (1984) noted that such relationship could create an epidemiologically significant concept. It meant that the diseases occurred more severe and endemic unless control measures were immediately applied. Zimmermann and Granata (2002) explained once the wounded part of cacti plants was infected, disease development in the tissue was very rapid as the biochemical characteristics of their juicy cells were appropriate for the growth conditions of various biotic agents.

Statistical analysis showed that necrotic spot disease took place maximum in Malacca, with mean of incidence and severity level around 72.00% and 30.00%, respectively. In contrast, the minimum occurrence of disease was found on dragon fruit crops which were planted far away from infected orchards in Terengganu, i.e. around 13.60% and 4.00% for mean of DI and DS, respectively (Table 1).

Neither with climatic nor cultural factors had significant the Pearson coefficient (Table 2). The incidence and severity of disease weakly correlated with those factors indicated by both small negative and positive of r values. Higher coefficient correlations were shown by correlation of disease prevalence with age of crops and acreage of orchard. These results explained that disease frequently occurred and more severe on older plants as well as on larger plantation.

CONCLUSION

Necrotic spot disease has been widely detected in dragon fruit-growing areas in Peninsular Malaysia with necrotic small mottle or spot which later turned orange and rotted symptomps. The spindle-shaped inclusion bodies of virus, which was then suspected as *Cactus virus X*, have been observed with TEM technique. The most severe disease significantly occurred in Malacca, while the less occurrence was recorded in Terengganu. No considerable correlation

was found between disease prevalence either with weather or cultural factors. Further study on the transmission of virus and disease-resistant variety of dragon fruit is important to be carried out in order to monitor the spread of disease and to develop the integrated disease management strategies on dragon fruit in Peninsular Malaysia.

ACKNOWLEDGEMENT

The authors would like to acknowledge and thank the Ministry of Higher Education, Malaysia for funding this research under Fundamental Research Grant Scheme (FRGS) 01-01-07-094FR.

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