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ORIGINAL ARTICLE

Chili (*Capsicum annuum* L.) genotypes resistant to *Pepper yellow leaf curl Thailand virus* (PepYLCTHV)

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Abstract

Pepper yellow leaf curl Thailand virus (PepYLCTHV) causes leaf curl disease in chili production regions of the tropics and subtropics. Information on PepYLCTHV disease severity and resistance in chili pepper is still limited in Thailand. This study reports PepYLCTHV disease severity through graft inoculation and selection of single resistant plants for use in a chili breeding program. Twenty-one chili genotypes consisting of the local cultivar (5) collected from Thailand, breeding lines (9) developed at Khon Kaen University (KKU), Thailand and improved lines (7) obtained from the World Vegetable Center, Taiwan were used in this study. Forty-five-day-old seedlings of all the genotypes were graft inoculated with PepYLCTHV in a randomized complete block design (RCBD) with three replications and 10 plants per replication and kept in a plastic net house. Disease symptoms were scored at 20, 27, 34, 41 48, and 55 days after graft/inoculation (DAI). Disease severity was visually recorded using 0–5 scores. Results showed that the disease severity of 21 chili genotypes significantly differed at 48 days after grafting. High resistance and stability were shown by 9853-123 genotypes. Two genotypes, PSP11-7 and PSP11-10-1, showed resistant reaction with disease severity scores of 1.9 and 1.8, respectively. However, among 21 chili genotypes or 630 grafted plants, 302 plants were successfully grafted inoculated plants. Therefore, from the results of this work, highly resistant plants (69 single plants) can be selected, selfed and advanced for breeding.

Keywords: artificial inoculation, begomovirus, genetic resistance, graft inoculation, pure lines

Introduction

Chili (*Capsicum annuum* L.) belongs to the genus *Capsicum*, which is unique among all plant genuses by producing typically pungent fruits. The pungency is due to secondary metabolites called capsaicinoids which fruits of genus *Capsicum* synthesize. Chili is a commercial crop for small-scale producers in Asia, Africa, and America. In Thailand, chili is an important commercial crop cultivated by smallholder farmers on more than a hundred thousand (101,280) hectares during rainy and dry seasons (FAO 2017). Chili production under open field conditions is affected by several abiotic and biotic stresses, especially diseases caused by fungi, bacteria, nematodes and viruses. The *Pepper yellow leaf curl virus* (PepYLCV) transmitted by whitefly (*Bemisia tabaci*) is a begomovirus. The PepYLCV causing leaf curl disease in chili is a severe production

constraint of peppers (hot and sweet) in tropical and sub-tropical regions (Tsai *et al.* 2006; Sakata *et al.* 2008; Shih *et al.* 2010; Tsai *et al.* 2011; Chiemsombat *et al.* 2018). In Thailand, during the dry season, leaf curl disease is the primary production constraint, and the disease incidence can reach up to 100% (Fig. 1) when virus infection starts at seedling or early plant growth stages. The management of this disease through host plant resistance remains a cost-effective, safe environmental, and human health strategy. In Thailand, begomovirus resistant traits have become mandatory for the formal release of chili varieties ("must-have trait"). Hence, the development of PepYLCV-resistance breeding in Thailand has become a priority.

Among different species of PepYLCV, *Pepper yellow leaf curl Kanchanaburi virus* (PepYLCKaV) and *Pepper yellow leaf curl Thailand virus* (PepYLCTHV) are the most prevalent, aggressive and problematic in Thailand (Chiemsombat *et al.* 2018). PepYLCV resistance breeding has been challenging mainly because of the lack of resistant germplasm against specific virus species (Barchenger *et al.* 2019). PepYLCV resistant sources have been identified and reported from India (Kumar *et al.* 2006; Kumar *et al.* 2011; Rai *et al.* 2014) and, most recently, from Taiwan (Barchenger *et al.* 2019). The Khon Kaen University (KKU), Khon Kaen, Thailand, also screened a few chili germplasms in a net house by releasing viruliferous whitefly harboring PepYLCTHV. Four resistant genotypes were identified (Sangsotkaew *et al.* 2018). However, the screening results based on viruliferous whitefly release (free choice) might have been influenced by the preference or non-preference of whiteflies for specific genotypes. Thus, it could not explain the resistance mechanism (antixenosis vs. antibiosis) in the identified resistant genotypes. Hence, we screened a set of potential PepYLCTHV resistant genotypes through the graft inoculation method. The results have been discussed in terms of their possible utilization to develop a resistance breeding program.

Materials and Methods

Plant materials

Twenty-one chili genotypes were purified by two successive generations of selfing in 2017 and 2018, and selfed genotypes were screened against PepYLCTHV isolate of the virus along with resistance (9853-123) and susceptible (KM-P13001-4) checks (Table 2). The screening experiment was conducted in a greenhouse from April to August 2019 at the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

Inoculation and disease evaluation

A highly virulent and purified PepYLCTHV collected from Kasetsart University (Kamphaeng Saen Campus, Nakorn Pathom), Thailand, was maintained on plants of a susceptible line (KM-P13001-4) and used in this study. The seeds of all the genotypes were sown in a peat moss plastic tray with 104 holds, and 45-day-old seedlings were transplanted into plastic plots size 19 cm diameter, 14 cm height. Seven days after transplanting into the pots, seedlings were grafted with an infected branch of KM-P13001-4 harboring PepYLCTHV isolate. Each grafted seedling was covered with a plastic bag to maintain the moisture and room temperature for 20 days or until a new shoot appeared. For each genotype, 30 seedlings were grafted, and the experiment was designed as a randomized complete block design (RCBD) with three replications and 10 plants per genotype. The disease severity was scored at 20, 27, 34, 41, 48, and 55 days after graft inoculation (DAI) as six severity score levels 0-5 (Table 3; Fig. 2). Each week the means of disease severity were analyzed by using Statistic 8 (V.2) developed by the United States Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS).

Table 1. Reports of artificial inoculation against begomovirus

Name of virus	Inoculation method	Resistant genotype	Reference
Chili leaf curl virus (ChiLCV)	graft inoculation	GKC-29, BS-35, EC-497636	Kumar *et al.* 2006
Mixed of pepper huasteco yellow vein virus geminivirus (PHYVV) and pepper golden mosaic virus (PepGMV)	graft inoculation	BG-3821	Anaya-Lopez *et al.* 2003
Chili leaf curl virus (ChiLCV)	graft inoculation	Puri Red and Puri Orange varieties	Mishra *et al.* 1963
Pepper leaf curl Thailand virus (PepYLCTHV)	viruliferous whiteflies inoculation	9852-123	Barchenger *et al.* 2019
Pepper leaf curl Thailand virus (PepYLCTHV)	viruliferous whiteflies inoculation	Perennial HDV, PSP-11, KR-B / NP-46-A, PBC145	Sangsotkaew *et al.* 2018
Chilli leaf curl virus (ChiLCV)	viruliferous whiteflies inoculation	Pusa Jwala, Surya Mukhi, Loungi	Kumar *et al.* 1999

Fig. 1. The severity (100%) of begomovirus in chili pepper production fields in Kanchanaburi Province of Thailand: A – the begomovirus infected plants, B – the healthy plants

Table 2. Chili genotypes used for screening against *Pepper yellow leaf curl Thailand virus* (PepYLCTHV)

S.N.	Genotype	Type*	Source	S.N.	Genotype	Type*	Source
1	PP0237-7508	IL	WorldVeg	12	Yodsonkem 80	LC-T	KKU
2	PP0437-7510	IL	WorldVeg	13	KM-P13001-4	IL (SC)	KKU
3	PP0537-7504	IL	WorldVeg	14	PSP11-3	IL	KKU
4	PP9955-15	IL	WorldVeg	15	PSP11-4	IL	KKU
5	PP0537-7559	IL	WorldVeg	16	PSP11-7	IL	KKU
6	PP0537-7541	IL	WorldVeg	17	PSP11-8-1	IL	KKU
7	9853-123	IL (RC)	WorldVeg	18	PSP11-8-2	IL	KKU
8	Huareua-7	LC-T	Srisaket	19	PSP11-10-1	IL	KKU
9	Huareua-12	LC-T	Srisaket	20	PSP11-10-2	IL	KKU
10	Kaekdum	LC-T	Supanburi	21	PSP11-11	IL	KKU
11	Hromsuphan	LC-T	Supanburi				

*RC = resistant check; SC = susceptible check; IL = improved line; LC = local cultivar from Thailand; WorldVeg = World Vegetable Center, Taiwan; KKU = Khon Kaen University (KKU), Thailand

Table 3. Disease severity score and disease response symptom description and disease response

Score	Symptom	Disease response
0	No visible symptoms on leaves	HR
1	0–5% curling and clearing of upper leaves	R
2	6–25% curling and clearing of leaves, and swelling of veins	MR
3	26–50% curling, puckering and yellowing of leaves and swelling of veins	MS
4	51–75% leaf curling and stunted plant growth and blistering of internodes	S
5	more than 75% curling and deformed small leaves, stunted plant growth without flowering	HS

Source: Adapted from Kumar *et al.* (2006)
HR = highly resistant, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, HS = highly susceptible

Verification of viral genome

At 55 DAI, 302 plants, which had successful grafting were examined for the presence or absence of the PepYLCTHV genome using begomovirus universal primers (Tsai *et al.* 2011) (Table 4). Total DNA was extracted from the young leaves. The polymerase chain reaction (PCR) was performed in a 40 µl reaction mixture containing 10 µl genomic DNA, 0.20 µl of Taq DNA polymerase, 5 µl of 10× PCR buffer, 4 µl of 10 mM dNTPs, 2.5 µl of 50 mM $MgCl_2$, 26.3 µl of H_2O and 1 µl of 10 µM of forward and reverse primers. The PCR program was as follows: initial denaturation

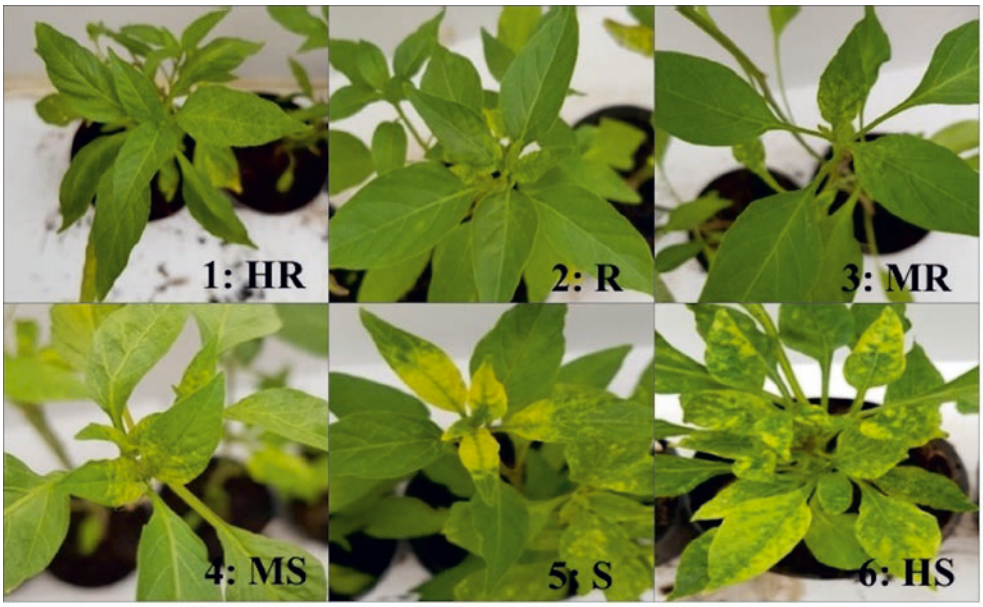

Fig. 2. Pepper yellow leaf curl virus (PepYLCV) scores in chili seedling stage. 1 – HR (highly resistant); 2 – R (resistant); 3 – MR (moderately resistant); 4 – MS (moderately susceptible); 5 – S (susceptible) and 6 – HS (highly susceptible)

Table 4. The sequence of universal primers associated with begomovirus

Marker	Primer sequence	Reference
PAL1v1978	5′-GCATCTGCAGGCCCACATBGTYTTHCCNGT-3′	Tsai *et al.* (2011)
PAR1c715H	5′-GATTCTGCAGTTDATRTTHTCRTCCATCCA-3′	Tsai *et al.* (2011)

at 94°C for 1 min; 30 cycles of amplification each consisting of 94°C for 30 s, 55°C for 30 s, and 72°C for 80 s; and a final extension period at 72°C for 10 min (Tsai *et al.* 2011). The PCR products were electrophoresed on 1.2% agarose gel, stained with ethidium bromide (5.0 g · 100 ml^{-1}) and visualized and documented using Alpha Imager 3,400 gel documentation system (Alpha Innotech, USA).

Results and Discussion

Plants of 21 chili genotypes were successfully screened using a virulent virus isolate of PepYLCTHV from Thailand by graft inoculation. Susceptible chili pepper genotypes in response to PepYLCTHV showed symptoms at an early date, while the resistant genotypes had no symptoms. However, the disease response was significantly different at 48 and 55 days after inoculation.

Resistance to PepYLCTHV and disease progress

The experimental plants were evaluated for disease score at six time intervals after graft inoculation and after final evaluation, chili genotypes were categorized into five different groups (Table 5). At 20 days after inoculation/grafting (DAI), all 21 genotypes were asymptomatic (highly resistant) and disease severity ranged from 0 to 0.8 on a 0–5 points rating scale (Table 5). Mild leaf curling symptoms started appearing on 27 DAI. However, at this stage, all genotypes were asymptomatic with the severity score ranging from 0 to 1.9. During the third evaluation (34 DAI), one genotype (9853-123) was highly resistant, while ten and nine chili genotypes showed resistant and moderately resistant responses, respectively (Table 5). After 55 DAI, only one genotype (9853-123) was highly resistant, and four genotypes (PSP11-3, PSP11-7, PSP11-10-1, PSP11-10-2) showed resistant reactions (Table 5, Fig. 3). As expected, 9853-123 did not develop symptoms on young and mature leaves even at 55 DAI. The second group included two resistant genotypes (PSP11-7 and PSP11-10-1) consisting of improved lines from KKU, Thailand. Five genotypes, PP0237-7508, PP0537-7504, Huareua-12, PSP11-3, and PSP11-10-2, were moderately resistant (Table 5). Nine genotypes (PP0437-7510, PP9955-15, PP0537-7541, Huareua-7, Hromsuphan, Yodsonkem 80, PSP11-4, PSP11-8-1, PSP11-11) were recorded as being moderately susceptible. Three genotypes PP0537-7559, Kaekdum, and PSP11-8-2) and one

Table 5. *Pepper yellow leaf curl Thailand virus* (PepYLCTHV) disease reactions of 21 genotypes at different graft inoculation stages

Genotypes	Disease severity						Response
	20 DAI	27 DAI	34 DAI	41 DAI	48 DAI	55 DAI	55 DAI
PP0237-7508	0.8	1.4	1.9	2.4	2.6	2.6	MR
PP0437-7510	0	0.6	2	2.2	3	3.2	MS
PP0537-7504	0.2	1.3	1.9	2.5	2.8	2.8	MR
PP9955-15	0.3	1.7	2.6	3.4	3.7	3.7	MS
PP0537-7559	0.3	1.2	2.7	3	3.8	4	S
PP0537-7541	0	0.9	1.7	2.2	2.9	3.1	MS
9853-123 (RC)	0	0	0	0	0	0	HR
Huareua-7	0	1.1	2	3	3.7	3.8	MS
Huareua-12	0	0.9	1.6	2.4	2.6	2.7	MR
Kaekdum	0.5	1.9	3	4	4.4	4.5	S
Hromsuphan	0.3	1.1	2	2.7	3.3	3.3	MS
Yodsonkem 80	0.2	0.5	1.9	2.4	2.7	3.1	MS
PSP11-4 (SC)	0	1.4	2.9	3.9	4.9	5	HS
PSP11-3	0.2	0.8	1.4	1.8	2.1	2.1	MR
PSP11-4	0.5	1.5	2.1	2.3	3.1	3.2	MS
PSP11-7	0.5	0.7	1.1	1.3	1.8	1.9	R
PSP11-8-1	0	1.9	2.5	3.1	3.5	3.8	MS
PSP11-8-2	0	1.4	2.9	3.6	4.5	4.5	S
PSP11-10-1	0.1	0.4	1.2	1.2	1.6	1.8	R
PSP11-10-2	0.2	0.9	1.4	1.8	2.3	2.3	MR
PSP11-11	0	0.9	1.6	2.3	3	3.2	MS
Mean	0.2	1.07	1.92	2.45	2.97	3.08	
F-test	ns	ns	ns	ns	**	**	

DAI = days after inoculation (grafting); HR = highly resistant, HS = highly susceptible, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible; ns = not significant; $^{**}p < 0.01$

genotype (KM-P13001-4) were susceptible and highly susceptible, respectively, during the experiments (Table 5, Fig. 4). Among all the genotypes evaluated, only 9853-123, from WorldVeg, Taiwan, had a stable, highly resistant reaction. The plants of genotype PSP11 selected from KKU, Thailand, were segregated into highly

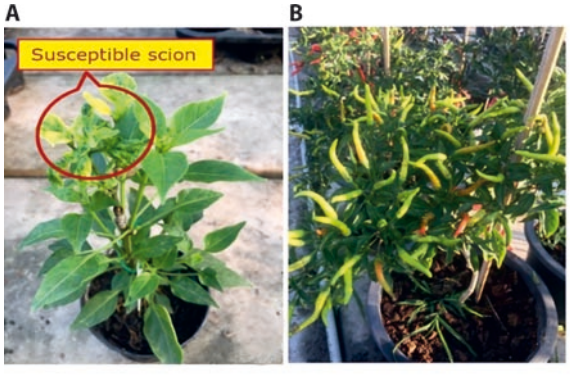

Fig. 3. Plants of PSP11: symptomless resistant at 34 DAI with susceptible scion of KM-P130031-4 (A) symptomless, mature fruits (B)

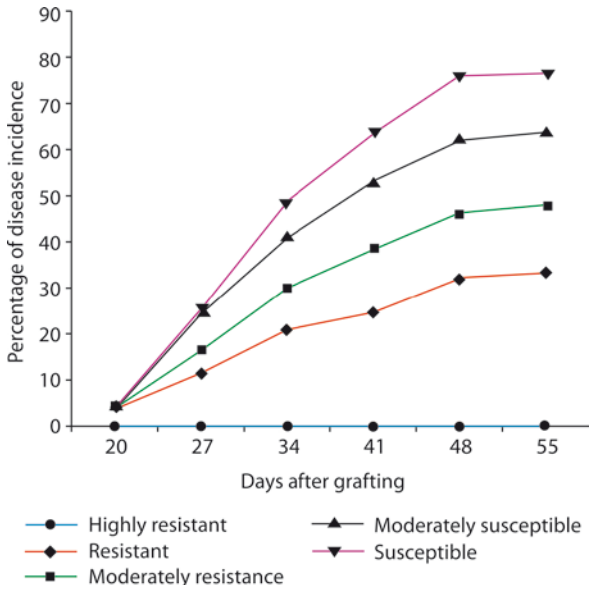

Fig. 4. The disease progress curves of five disease response groups at 20, 27, 34, 41, 48, and 55 days after grafting with scion containing PepYLCTHV

resistant to highly susceptible plants. In recent research (Barchenger *et al.* 2019), among 98 *Capsicum* germplasms inoculated by viruliferous whiteflies at two locations in Thailand, resistant check (9853-123) used in this study was the only highly resistant source to PepYLCKaV and PepYLCTHV strains (Barchenger *et al.* 2019). Therefore, the results also suggest that 9853-123 contains the resistance mechanism of non-preference whitefly inoculation (Barchenger *et al.* 2019) and induced the resistance mechanism. In addition, some lines of PSP11 genotypes were segregated to resistant and susceptible, because of there are not purified in the resistance to PepYLCTHV disease trait like the segregation (resistant and susceptible) of Loungi cultivars, which it was reported resistant to leaf curl virus (Kumar *et al.* 1999). Many reports suggest that some other accessions of Indian origin consistently showed resistant reactions. For instance, PBC145 is an erect fruited Indian accession known for its multiple resistant traits (Srivastava *et al.* 2017).

Begomovirus verification

Based on the disease reaction to PepYLCTHV at 55 DAI, we observed that the genotypes of successfully grafted plants segregated. Hence 69 highly resistant (score 0) plants from 19 genotypes were tested for the presence of viral genome (Table 6). Among highly resistant plants (69), 32 plants were devoid of the viral genome, while the remaining 37 plants had a viral genome (Table 6). Selfed seeds of these 69 plants were collected for further advancement. The absence of a viral genome in highly resistant plants could be due to the resistance mechanism suppressing viral genome replication or movement of viruses in the phloem (Verlaan *et al.* 2013). The viral genome was present in all the susceptible check plants (KM-P13001-4) examined only after 7 DAI. Thus, KM-P13001-4 could be considered as an excellent susceptible check in Thailand for future study. Inheritance of resistance to begomovirus in chili has been found to be variable depending on the resistant source, virus isolate, and phenotyping method used. In BG3821, two recessive resistant genes

Table 6. Disease score, presence/absence of viral genome, disease incidence, and responses within genotype at 54 days after inoculation

Genotypes	Number of plant response to different disease scores*							Number of plant virus detection		
	0(+)	0(-)	1(+)	2(+)	3(+)	4(+)	5(+)	undectable	detectable	percentage of grafted [%]
PP0237-7508	1	1	0	0	1	2	4	1	8	30.00
PP0437-7510	2	0	0	0	3	1	5	0	11	36.67
PP0537-7504	0	6	2	2	0	3	10	6	17	76.67
PP9955-15	2	0	5	2	0	0	13	0	22	73.33
PP0537-7559	0	0	0	0	0	2	5	0	7	23.33
PP0537-7541	4	1	3	1	0	2	10	1	20	70.00
9853-123 (RC)	2	2	0	0	0	0	0	2	4	13.33
Huareua-7	1	3	3	0	0	0	11	3	28	60.00
Huareua-12	1	4	0	1	1	2	6	4	14	50.00
Kaekdum	1	0	1	0	0	1	14	0	17	56.67
Hromsuphan	2	1	2	1	0	0	8	1	23	46.67
Yodsonkem 80	3	2	0	0	1	4	7	2	17	56.67
KM-P13001-4 (SC)	0	0	0	0	0	0	14	0	14	46.67
PSP11-3	1	6	3	4	1	2	5	6	22	73.33
PSP11-4	4	0	2	1	0	1	9	0	17	56.67
PSP11-7	3	2	2	1	2	0	4	2	14	46.67
PSP11-8-1	0	1	0	1	1	0	5	1	7	26.67
PSP11-8-2	1	0	0	0	0	2	11	0	14	46.67
PSP11-10-1	4	1	1	0	0	2	1	1	9	30.00
PSP11-10-2	3	0	1	0	1	1	3	0	8	30.00
PSP11-11	2	2	1	2	1	2	7	2	15	56.67
Total	37	32	26	16	12	27	152	32	271	

* 0(+) and 0(-) = disease score 0 with presence and absence of viral genome, respectively

(Anaya *et al.* 2003) and in Bhut Jolokia monogenic recessive control (Rai *et al.* 2014) of PepLCV resistance has been reported. Therefore, from the results of this work, individual resistant plants such as PP0237-7508, PP0537-7504, PP0537-7541, Huareua-7, Huareua-12, Hromsuphan, Yodsonkem 80, PSP-11, and 9852-123 which are based on their high resistance to moderately resistant genotypes were selected and selfed for crossing with an elite line for developing mapping population, inheritance study, and development of markers for resistance.

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