Concept Proposal
Collaborative project between
Asia and Pacific Seed Alliance LTD (APSA) and
King Mongkut's Institute of Technology Ladkrabang (KMITL)
National Omics Center (NOC) National Science and Technology Development Agency (NSTDA)

Project Title: Identification of single nucleotide polymorphism (SNP) markers associated with resistance to pepper yellow leaf curl virus in chili pepper

Investigators:

Principal Investigator

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Project Duration: 3 Years (To start from June 1, 2022 – May 31, 2025)

Funding requested from APSA (US$)	53,000	Minimum of 5 companies with US$ 10,600 per company
		Maximum of 10 companies with US$ 5,300 per company

Total budget 3,069,400 Thai Baht

Contribution APSA 50%: NSTDA 50%

- APSA in-cash: 1,530,100 Thai Baht (include 7% vat)
- NSTDA in-kind: 1,539,300 Thai Baht

Objective

- To identify single nucleotide polymorphism (SNP) markers associated with resistance to pepper yellow leaf curl virus in chilli pepper

Research project output

- SNP markers linked to pepper yellow leaf curl virus resistance that can be used for marker-assisted breeding

Abstract

The whitefly transmitted *Begomovirus*, which causes pepper yellow leaf curl (PepYLC), is among the most devastating chilli pepper-infecting viruses in Thailand and other tropical regions. The disease can lead to as high as 100% yield loss. Management of begomoviruses has

been primarily through the use of insecticides against the whitefly vector; however, it is only partially effective and quite costly for farmers. Sources of resistance to *Begomovirus* have been reported, including the *Capsicum annuum* psp11 line available at the World Vegetable Center (AVRDC), which we obtained and self-fertilized several times to generate a line called Pep6. Intriguingly, we observed a segregation in *Begomovirus* resistance in every generation of Pep6 self progeny. Since Pep6 has been self-fertilized for several generations, we expected the progeny to be nearly isogenic except for the regions linked to *Begomovirus* resistance. Comparison of genomic sequences from susceptible and resistant self progeny from the same generation should reveal single nucleotide polymorphism (SNP) markers associated with resistant phenotype. We propose to carry out a whole genome sequencing of three types of Pep6 F_6 self progeny: (1) susceptible to *Begomovirus*, (2) resistant to *Begomovirus* with the presence of virus Detected *in planta* (by PCR) and (3) resistant to *Begomovirus* with no virus detected *in planta*. We expect the regions that are polymorphic among susceptible and resistant progeny to be linked to the resistant phenotype. We would also like to generate a *Begomovirus*-resistant backcross progeny derived from a commercial line (Jindanil) and Pep6 and perform a whole genome sequence to identify SNP markers that are linked to *Begomovirus* resistance.

Background

Chili pepper belongs to the Capsicum genus, including six main cultivated species, *C. annuum, C. baccatum, C. chinense, C. frutescens, C. pubescens* and *C. assamicum*. It is economic crop of the World, because it is utilized in several ways: fresh vegetables, spices, pigments, and medical supplies. The world's largest producer is China, followed by the Mexico. While for dry pepper, Asia and Africa are the main producers contributing to the 70.3 and 21.2%, respectively. India is the largest producer, followed by Thailand (FAOSTAT 2017). Chili production is affected by abiotic and biotic stresses. Pepper yellow leaf cure virus (PepYLCV) is one of the most serious problems of the chili production in South and South East Asian countries including Thailand. The disease was first reported in Indonesia and spread thought in subtropics and tropics. In Thailand, PepYLCV has been observed in Kanchanaburi province, Thailand since 1995. The disease plants showed striking yellow mosaic of leaves, leaf distortion and small size leaves at plant apices. Fruits became pale green or yellow and were deformed (Hidayat et al., 1999: Chiemsombat et al., 2018). Management of begomoviruses has been based primarily on insecticides against the whitefly vector. However, the use of insecticides has been found to be only partially effective, costly for producers, and represents a hazard to farmers, consumers, and the environment (Borah and Dasgupta, 2012), while limiting export potential through presence of pesticide residue. Farmers often apply the insecticides after seeing early disease symptoms, by which time the whiteflies have already transmitted the virus to other plants (Kenyon et al., 2014). An effective alternative to harmful insecticide applications for management of Begomovirus is the development of resistant cultivars (Shankarappa et al., 2008).

Genetic mapping is an important method for positioning genes of interest in genome as well as identifying quantitative trait loci (QTLs) responsible for natural phenotypic variation (Xu et al. 2017). Recently, molecular breeding techniques such as QTL mapping and introgression, identification of causative genes, and molecular marker development have been utilized for breeding enhancement (Mohan and Paran 2019). Molecular markers, which can be

classified into biochemical and DNA markers, indicate a visible phenotype or fragment of DNA that is associated with a certain location within the genome (Kumar 1999).

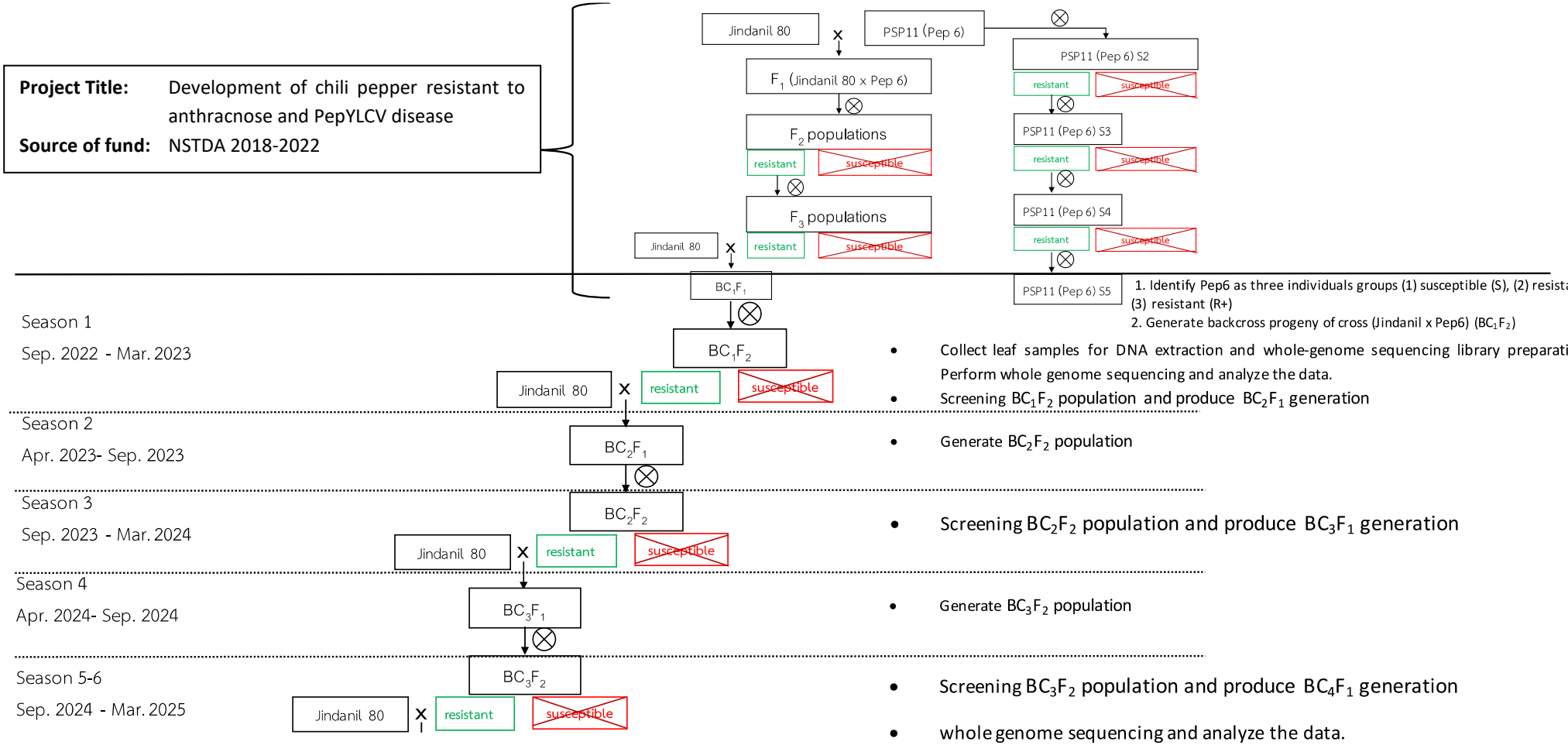
Sources of resistance to Begomovirus in chile pepper have been reported, including the *C. chinense* line BG-3821 in Mexico (Anaya-Lopez et al., 2003) and 'Bhut Jolokia' in India (Adluri et al., 2017). The *C. annuum* lines DLS-Sel-10, WBC-Sel-5, WorldVeg Pepper Breeding Collection (PBC) 142, PBC 145, PBC 345 (Srivastava et al., 2015, 2017), PBC 143, PBC 144, PBC 149, PBC 495, VI012005 (Kenyon et al., 2014), GKC-29, BS-35, EC-497636 (Kumar et al., 2006), and 'Kalyanpur Chanchal' (Singh et al., 2016) have been reported to be resistant. Wang and Bosland (2016) have been report in Bhut Jolokia, resistance to PepYLCV was under control of a single, recessive gene. Koeda et al. (2021) reported the BaPep-5 line was resistant to pepper yellow leaf curl Aceh virus (PepYLCAV) and pepper yellow leaf curl Indonesia virus (PepYLCIV) and identified resistance gene which was single recessive locus, as pepy-1 gene. However, in our previous experiment we found the genetic resistant to pepper yellow leaf curl Thailand virus from PSP11 and 9852-123 lines as given coded Pep6 and Pep12, respectively. These lines will be served as important genetic resistant source for improving resistant commercial varieties. For plant breeding, molecular markers can substantially improve selection efficiency and reduce breeding time compared to conventional breeding (Lee 2019).

The advent of DNA-based genetic markers enabled plant breeders to accelerate their breeding programs through the utilization of marker-assisted selections. In the past two decades, several types of molecular markers have been developed (restriction fragment length polymorphisms, random amplified polymorphic DNAs, simple sequence repeats) and used in the construction of genetic linkage maps and various phylogenetic studies. Recently, attention has been geared toward the use of single nucleotide polymorphisms (SNPs) as genetic markers. The ubiquity of SNPs in eukaryotic genome and their usefulness as genetic markers has been well established over the last decade. SNP markers typically occur at frequencies of one per ~100-500 bp in plant genomes, depending on the species, e.g. 1 SNP/490 bp in soybean (Choi et al., 2007) and 1 SNP/540 bp in pea (Leonforte et al., 2013).

With rapid advancement in sequencing throughput together with an overall decrease in sequencing cost, next generation sequencing technologies have been applied to SNP identification in various plant species (Ganal et al., 2009). Here, we propose to identify SNP markers linked to PepYLCV resistance in Pep6 self-progeny using bulk segregant analysis. Having gone through multiple rounds self-fertilization, the Pep6 self-progeny should have relatively homogeneous genetic background except the region associated with the segregating PepYLCV resistance. We will employ the short-read sequencing platform, MGIseq RS-2000 to obtain shotgun whole genome sequences from three types of Pep6 F_6 self progeny: (1) susceptible to *Begomovirus*, (2) resistant to *Begomovirus* with the presence of virus Detected *in planta* (by PCR) and (3) resistant to *Begomovirus* with no virus detected *in planta*. High-quality reads will be mapped to the *C. annuum* genome sequence, and SNP markers will be called in each line. Bioinformatic tools will be employed to perform the marker-trait association analysis. SNPs linked to PepYLCV resistance will be identified. Moreover, we will scan chromosome segments associated with resistance for candidate genes that may be conferring PepYLCV resistance in chili pepper. A similar process will be carried out for Jindanil x Pep6 backcross progeny. We expect to uncover the same genomic regions linked to PepYLCV resistance.

Project Title: Development of chili pepper resistant to anthracnose and PepYLCV disease

Source of fund: NSTDA 2018-2022



1. Identify Pep6 as three individuals groups (1) susceptible (S), (2) resistant (R+) and (3) resistant (R+)
2. Generate backcross progeny of cross (Jindanil x Pep6) (BC_1F_2)

- Collect leaf samples for DNA extraction and whole-genome sequencing library preparation. Perform whole genome sequencing and analyze the data.
- Screening BC_1F_2 population and produce BC_2F_1 generation
- Generate BC_2F_2 population
- Screening BC_2F_2 population and produce BC_3F_1 generation
- Generate BC_3F_2 population
- Screening BC_3F_2 population and produce BC_4F_1 generation
- whole genome sequencing and analyze the data.
- Compare the regions and polymorphic SNP markers identified as “linked to Begomovirus resistance” from the Pep6 self progeny and those from Jindanil x Pep6 backcross progeny.

Research Plan

Activity	Month						Persons Responsible
	1-6	7-12	13-18	19-24	25-30	31-36	
1. Germinate and phenotype F_6 progeny to identify two individuals from each type (1) susceptible to *Begomovirus*, (2) resistant to *Begomovirus* with the presence of virus detected *in planta* (by RT-PCR) and (3) resistant to *Begomovirus* with no virus detected *in planta.* 2. Generate Jindanil x Pep6 backcross progeny	X X	X	X				KMITL, NSTDA
3. Collect leaf samples for DNA extraction and whole-genome sequencing library preparation. Perform whole genome sequencing and analyze the data. 4. Generate Jindanil x Pep6 backcross progeny (BC_2F_2).		X X					NSTDA, KMITL
5. Phenotype backcross BC_2F_2 progenies to identify susceptible and resistant individuals to be used for whole genome sequencing 6. Generate Jindanil x Pep6 backcross progeny (BC_3F_1)			X X				KMITL
7. Generate Jindanil x Pep6 backcross progeny (BC_3F_2)				X			KMITL
8. Phenotype backcross BC_3F_2 progenies to identify susceptible and resistant individuals to be used for whole genome sequencing 9. Generate Jindanil x Pep6 backcross progeny (BC_4F_1)					X X		KMITL

Activity	Month						Persons Responsible
	1-6	7-12	13-18	19-24	25-30	31-36	
10. Collect leaf samples for DNA extraction and whole-genome sequencing library preparation. Perform whole genome sequencing and analyze the data. 11. Compare the regions and polymorphic SNP markers identified as "linked to *Begomovirus* resistance" from the Pep6 self progeny and those from Jindanil x Pep6 backcross progeny.						**X** **X**	KMITL, NSTDA

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