Research Proposal:

Multi-location evaluation of chili lines carrying different combinations of *pvr* and *Cvr* genes for resistance to *Chili veinal mottle virus* (ChiVMV)

Proposal Summary

Project title	Multi-location evaluation of chili lines carrying different combinations of *pvr* and *Cvr* genes for resistance to *Chilli veinal mottle virus* (ChiVMV).
Main WorldVeg contact person	Mandy Lin (mandy.lin@worldveg.org)
Main WorldVeg scientists	Dr. Derek Barchenger (derek.barchenger@worldveg.org) Dr. Lawrence Kenyon (lawrence.kenyon@worldveg.org)
Project duration	2 years (1 March 2020 – 28 February 2022)
Estimate budget contribution per company (US$)*	**8,500 to 26,500**

***The range of budget contribution per company is calculated based on a number of companies showing interest to jointly fund the project, however, the final amount of the required contribution per company may be or may not be the same as indicated above as some companies may drop off.** The final amount of the required contribution will be announced once APSA confirms the companies' intention to sign the agreement.*

Objective

To assess the performance of advanced pepper breeding lines carrying different combinations of *Pvr* and *Cvr* genes in different locations in Asia where different pathotypes of pepper-infecting *Chilli veinal mottle virus* (ChiVMV) are predominant.

Background

Over the last 30 years, global chili (*Capsicum* sp.) consumption has increased 40-fold with ~25% of people consuming some form of pepper every day. Being a high-value crop, chili has immediate economic benefits for producers. According to the FAO, global production of chili was 56.2 million tonnes on an area of 4.5 million hectares in 2016 (FAOSTAT, 2016). Approximately 65% of chili is produced in Asia, with India (1.5 million tonnes) among the top 5 largest producers (FAOSTAT, 2016). The primary limitations to increased chili productivity and quality are biotic and abiotic stresses. In addition to increased consumer demand for chili, the past 3 decades has seen the number of virus species infecting chili as well as virus disease incidence considerably increase (Kenyon et al., 2014).

The genus *Potyvirus* (Family Potyviridae) is a large group of plant viruses that cause disease in a wide range of plant species (Kenyon et al., 2014). Potyviruses have a single-stranded, linear, positive-sense RNA genomes of ~9.7 kb, and are transmitted by one or more aphid species, or transmitted mechanically and by grafting (Kenyon et al., 2014). *Chilli veinal mottle virus* (ChiVMV) is probably the second most prevalent chili-infecting virus across South, East, and Southeast Asia (Tsai et al., 2008), after members of the genus *Begomovirus*. Characteristic symptoms of ChiVMV infection are leaf mottle and dark green vein banding, and leaves may be small and distorted. If

plants are infected when young, they may become stunted, with dark green streaks on their stems and branches and often most of the flowers will drop, resulting in considerable yield loss (Kenyon et al., 2014). In open field conditions, ChiVMV and other viruses such as *Cucumber mosaic virus* (CMV) (Naresh et al., 2016), members of *Begomovirus,* and *Pepper veinal mottle virus* (PVMV) (Cheng et al. 2009) are often found to co-infect the chili crop, which makes it difficult to correctly identify and therefore manage the disease and reduced the effectiveness of resistance (Kenyon et al., 2014). Management strategies for ChiVMV include controlling aphid vectors, limiting mechanical transmission on hands or implements, and planting resistant cultivars. The use of resistant cultivars is the most environmentally friendly and economical management strategy of viral diseases in chili.

The *Capsicum–Potyvirus* pathosystem has been well studied and used as a model reference for the study of similar pathosystems in other plant species. Resistance to potyviruses is associated with two major gene groups. First are those with recessive inheritance (*pvr1*/*pvr2*, *pvr5*, *pvr6*, and *pvr8*) and associated with a mutation of the host factor eIF4E or eIF(iso)4E. The second group of single-gene potyvirus resistance mechanisms comprises those with dominant inheritance patterns (*Pvr4*/*Pvr7* and *Pvr9*) and are usually associated with NLR cluster regions (Lee et al., 2017). One study using doubled haploid lines derived from a cross between 'Perennial' and 'Yolo Wonder' revealed that two independent dominant genes were required for ChiVMV resistance and that resistance was dominant in F_1 plants (Caranta and Palloix 1996). It has also been reported that *pvr6* in combination with $pvr1^1$ or $pvr1^2$ is required for resistance to ChiVMV, demonstrating an interaction between eIF4E and eIF(iso)4E with the ChiVMV viral protein genome-linked protein (VPg) (Hwang et al., 2009). Using ChiVMV-Bangalore isolate in India, Naresh et al. (2016) found that resistance in two of their sources (IHR 2451 and IHR 4503) was monogenic recessive. Recently, Lee et al. (2013) identified a novel dominant resistance gene, *Cvr1*, on chromosome 6, and developed associated molecular markers, although the markers were not closely positioned to the gene, with the CVMV3 marker being 3 cM away. Lee et al. (2017) further studied the inheritance patterns of new resistance genes and identified the oligogenic inherited *Cvr2-1* and *Cvr2-2* genes to be associated with resistance in some of their lines. The authors also identified a novel single recessive resistance gene, *cvr4*, although it was not mapped to a chromosome (Lee et al., 2017). The World Vegetable Center recently conducted targeted sequencing of the *Cvr1* region in our sources of ChiVMV resistance and developed a new molecular marker, CVMV3-SCAR, which is tightly associated with resistance in Taiwan in some of our sources of resistance (unpublished data).

Many factors are involved in resistance to ChiVMV, including the environment, vector-pressure, resistance genes in the host, and pathotype of the virus. Shah et al. (2011) identified at least two pathotypes of ChiVMV causing differential responses on different local and exotic chili genotypes in Pakistan, and preliminary findings at The World Vegetable Center indicate that there are several more different pathotypes of ChiVMV in different areas of South and Southeast Asia (Tsai et al., 2008). More research in this area is required to better understand the pathotype structure of ChiVMV in some of the major chili production regions, which will allow for more efficient resistance gene identification and the rapid development of more durable resistant cultivars.

Table 1. Summary of previously reported potyvirus resistance genes and loci associated with ChiVMV resistance

Resistance gene or locus	Inheritance mode	Chr	Allelism	Source
pvr1	Recessive	3		Kang et al. 2005; Kyle and Palloix 1997; Murphy et al. 1998; Yeam et al. 2005
pvr1[1]	Recessive	3	*pvr1*	Kang et al. 2005; Ruffel et al. 2002; Yeam et al. 2005
pvr1[2]	Recessive	3	*pvr2*	Caranta et al. 1997; Yeam et al. 2005
pvr3	Recessive	-		Kyle and Palliox 1997; Zitter and Cook 1973
Pvr4	Dominant	10	*Pvr7*	Dogimont et al. 1996; Kim et al. 2017
pvr5	Recessive	3	Likely *pvr1*	Kang et al. 2005
pvr6	Recessive	9		Caranta and Palloix, 1996; Hwang et al. 2009; Ruffel et al. 2006
Pvr7	Dominant	10	*Pvr4*	Grube et al. 2000
pvr8	Recessive	-		Dogimont et al. 1996
Pvr9	Dominant	6		Tran et al. 2015
Cvr1	Dominant	6		Lee et al. 2013
Cvr2-1	Dominant	6		Lee et al. 2017
Cvr2-2	Dominant	10		Lee et al. 2017
cvr4	Recessive	-		Lee et al. 2017

Methods/Activities

This project will take place over a 2 year period

Activity 1: Conduct collaborative trials of pepper lines carrying different combinations of *pvr* and *cvr* genes in different seed company managed sites at different locations in Asia where infection by ChiVMV is a major problem.

A set of 15 WorldVeg pepper advanced breeding lines representing different combinations *pvr1*, *Pvr4*, *pvr6*, and *Cvr1* has been identified as well as one susceptible check, which does not have any resistance genes (Table 2). Sufficient seed of these lines is available in the first year (2020) and has been cleared by BAPHIQ for distribution out of Taiwan to be able to establish at least ten field trials in APSA member managed field sites in different locations in Asia. Ideally the sites should be in diverse locations in different parts of Asia where infections with diverse ChiVMV pathotypes are prevalent in chili. Final choice of which sites will be used in each year of the project will be dependent on what sites are offered by project supporting APSA member companies and through discussion/negotiation at a project planning/steering group meeting.

In order to avoid symptom scoring bias the lines will be anonymized and coded before distribution to the participating seed companies. At each location the trial will be planted to the same design (as prescribed by WorldVeg), though the company managing a site should add two susceptible local check varieties, in addition to the WorldVeg susceptible check, and may add up to 6 of their own ChiVMV lines or hybrids. Each trial will be planned to be planted to be growing over the peak season for ChiVMV disease pressure locally. The company managing the site will be responsible for all cultural management of the trial and for assessing the trial for diseases and performance (growth habit, fruit type, yield, quality *etc*.). The plants in each trial on a regular basis (every 2 weeks) will be scored for ChiVMV disease symptom severity using a standard scale (1-6) provided by WorldVeg, and the scores will be sent to WorldVeg by e-mail for compiling and analysis to identify the most effective *pvr* and *Cvr* gene combinations for those areas. Dependent on the condition of each trial (as communicated by the local trial manager) and the availability of WorldVeg staff (and through negotiation/agreement in the project steering group) WorldVeg staff will visit some of the trials when ChiVMV disease is well established in order to observe the performance of the lines and make independent assessment of the diseases severity in each line. If the logistics permit, then these visits by WorldVeg staff will be used as an opportunity to open the visited trial to inspection by other project supporting APSA member companies (mini project field-day/workshops) so that they may see the performance of the lines in different locations and may interact with the WorldVeg scientists.

Activity 2. Identify the predominant ChiVMV pathotypes at each trial site.

Samples of each chili line showing symptoms of virus infection (from very mild to severe) from each field trial (set up in activity 1 above) will be collected and dried using the protocol provided by WorldVeg and then sent to WorldVeg-Taiwan (with the appropriate Taiwan Import Permit). Those samples collected in India will be sent to the WorldVeg South Asia Office in Hyderabad. Staff from WorldVeg HQ will travel to the South Asia office to conduct the analysis. At WorldVeg, each sample will be subject to Polymerase chain reaction (PCR) with universal ChiVMV primers to test for the presence of ChiVMV infection. Restriction digest analysis and/or sequencing will be used to identify which ChiVMV pathotype is present in selected PCR-positive samples. This is to determine which ChiVMV pathotype(s) is/are prevalent at each trial location, and to determine if pyramiding these resistances is useful because different *pvr* and *cvr* genes are effective against different ChiVMV pathotypes, or because there is an additive effect of resistance against all ChiVMV pathotypes, or a combination of these.

Activity 3. Seed production and further molecular evaluation of WorldVeg lines containing combinations of *pvr* and *Cvr* genes.

Sufficient seed of test lines for all the trials in both years has been produced at WorldVeg HQ and is available. Additional seed of each line will be produced at WorldVeg HQ for downstream distribution and testing. All lines will be tested using publicly available molecular markers associated with resistances to other key diseases such as *Colletotrichum* spp., *Phytophthora capsici*, *Ralstonia solanacearum*, other potviruses, tobamoviruses, as well as for sterile cytoplasm and presence of *Restorer-of-fertility* genes

Activity 4. Hybrid development and collaborative performance evaluation by WorldVeg and participating companies

Based on the results of activities 1 and 3 in 2020, selected hybridizations will be made among the best performing WorldVeg lines. Emphasis will be placed on highly resistant lines across the test

locations and presence of resistance genes to other diseases. Performance data (e.g. yield, fruit length and width, plant habit, etc) of the developed hybrid lines will be collected by WorldVeg and distributed to participating companies. APSA-consortium companies participating in this project will also be invited to observe and take their own notes on the performance of the hybrids developed during the WorldVeg-APSA Consortium workshop in 2021. However, no travel or accommodation costs for this will be provided by WorldVeg outside of what is covered in the Consortium agreement.

Table 2. WorldVeg breeding lines highly resistant to the Taiwan pathotype of ChiVMV containing different combination of *pvr* and *Cvr* genes based on presence of associated molecular markers selected for multi-location trials in 2020 and 2021.

Code	Other name	Phenotype (TW strain P714)	ELISA (TW strain P714)	Pvr1	Pvr4-EI2	pvr6	CVMV3	CVMV3 - SCAR
VI012287	VI012287	S	S	-	-	-	-	-
PBC518	VI037438	R	R	+	-	-	-	-
PBC1743	AVPP1812	R	R	-	+	-	-	-
PBC351	VI012253	R	R	-	-	+	-	-
PBC461	VI037542	R	R	+	-	-	+	-
PBC370	VI037606	R	R	+	-	-	-	+
PBC1735	AVPP1805	R	R	+	-	-	-	+
PBC1740	AVPP1809	R	R	-	+	-	-	+
PBC1742	AVPP1811	R	R	-	+	-	-	+
PBC569	VI046889	R	R	-	-	+	-	+
PBC1972	VI064760	R	R	+	-	+	+	-
PBC506	VI037529	R	R	+	-	+	+	-
PBC1739	AVPP1808	R	R	+	+	-	-	+
PBC1736	AVPP1806	R	R	+	+	+	-	+
PBC596	VI037558	R	R	+	+	+	-	+
PBC1738	AVPP1807	R	R	+	+	+	-	+

Deliverables

1. Comparable performance data for the latest WorldVeg breeding lines with different combinations of *pvr* and *Cvr* genes from different locations where different ChiVMV is prevalent.
2. Identification of the currently prevalent ChiVMV pathotypes at the different trial locations.
3. Performance evaluation of hybrids developed from lines identified as being highly-ChiVMV resistant containing different combination of *pvr* and *Cvr* genes and potentially other disease resistance genes.

Timeline of Activities

	Year 1		Year 2	
	1st half	**2nd half**	**1st half**	**2nd half**
Distribute seeds, experimental design, standardized scoring rubric, etc. from WorldVeg HQ to member companies				
Field trials conducted at member company locations across Asia and field visits				
ChiVMV isolates sent to WorldVeg HQ for characterization				
Analysis to characterize the ChiVMV pathotype provided by each member company				
Seed increase and line molecular evaluation of WorldVeg lines containing combinations of *pvr* and *Cvr* genes.				
Hybrid development based on presence of other resistance genes and performance in the field trials				
Hybrid trial during WorldVeg-APSA Consortium workshop				
Results compiled, final report developed and disseminated to all member companies				

Operational details

- A contract will be drafted and agreed upon, including all relevant parties involved.
- The Principal Investigator of the project at WorldVeg will be Dr. Derek Barchenger.
- Costs of the project will be paid by the participating APSA member companies. Each six months WorldVeg will send a statement of spending to APSA secretariat along with a request for funding for the following 6 months.
- The field trials will be set up and managed by the hosting APSA member company of each site using the design provided by WorldVeg and following the agreed workplan.
- The choice of field-trial sites and timing of each trial is as agreed at the APSA-WorldVeg 2019 workshop in Shanhua.
- Trials will be of a randomized complete block design (RCBD) – WorldVeg will provide the design layout including randomization for each trial site
- Susceptible checks and boundary plants should be planted out 2 weeks before the test plants.
- ChiVMV severity scoring should start at 2 weeks after transplanting and use the 1(Healthy) – 6(Very severe) scale [WorldVeg will provide color scoring cards] – all 12 plants of each test line in each block should be scored separately and the separate scores sent to WorldVeg by e-mail for analysis [WorldVeg will provide scoring guidelines (including what other data to collect) and template for recording scores]. Scoring should be carried out every

2 weeks. Pictures of plants with representative symptoms should be included, and incidence of other diseases should be recorded at each scoring.

- At the 6-week scoring the trial manager should discuss with WorldVeg if it is appropriate to collect leaf samples yet for ChiVMV identification. If approved 20-40 leaf samples each from a different plant and where possible from different levels of disease severity. Instructions on collecting and drying leaf samples with ChiVMV symptoms will be provided by WorldVeg. In India, dried samples should be sent to WorldVeg regional center in ICRISAT/Hyderabad for consolidating with others and sending to Taiwan (with import permit) for detection/identification. Samples from trials in other countries should be sent directly to Tainan quarantine station (with an appropriate import permit obtained by WorldVeg) for collection by WorldVeg.
- At WorldVeg the samples will first be tested for presence of ChiVMV by PCR with universal Potyvirus primers. Based on restriction digest patterns or short sequence reads, selected samples will be taken forward for full-length sequencing to determine what genotypes/species of ChiVMV are present at each site.

Results and Reporting

- The companies will be provided with a written report including all results obtained during the performance of the research every 12 months after the start of the project.
- At least once a year, a project meeting will be scheduled where progress and changes to the work plan will be discussed. If appropriate, this may coincide with a visit by WorldVeg staff to one of the company-managed field trials, or may be held at WorldVeg headquarters Taiwan and coincide with the WorldVeg-APSA Consortium workshop.
- A final report including all results will be provided to the companies within three months after the end of the project.

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