Research Proposal:

Chili Leaf Curl Disease in Asia: Diversity and Resistance

Proposal Summary

Project title	Chili Leaf Curl Disease in Asia: Diversity and resistance
Main WorldVeg contact person	Mandy Lin (mandy.lin@worldveg.org)
Main WorldVeg scientists	Dr. Lawrence Kenyon (lawrence.kenyon@worldveg.org) Dr. Derek Barchenger (derek.barchenger@worldveg.org)
Project duration	3 years (1 March 2020 – 28 February 2023)
Estimate budget contribution per company (US$)*	**10,000 to 22,500**

***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

In this project, we propose a multimodal approach as the most efficient and impactful strategy to tackle Chili Leaf Curl Disease in Asia, with the overall objective of expanding the boundaries of our understanding of the genetics of resistance in the host and the phylogeny and genetic recombination rates in the pathogen.

The specific objectives include 1) confirmation of WorldVeg resistance sources to new ChiLCD isolates, 2) identification of novel sources of resistance to ChiLCD in a biodiverse germplasm set, and 3) collection and phylogenetic characterization of the *Begomovirus* species infecting chili and other hosts across Asia.

Background

Consumer demand for chili (*Capsicum annuum*) has substantially increased over the past 30 years, especially for hot chili pepper. It has been estimated that chilis are consumed daily by approximately a quarter of the world's population (Smith et al., 2015). According to the FAO, global production of chili was 56.2 million tonnes on an area of 4.5 million hectares in 2016, with~65% of chili produced in Asia (FAOSTAT, 2016). Being a high value crop (DeWitt and Bosland, 1993), chili can have economic benefits for smallholder farmers, significantly increasing family income and socioeconomic mobility (Kahane et. al., 2013; Montes, 2010; Weinberger and Lumpkin, 2007).

The primarily limitations to increased chili productivity and quality are biotic and abiotic stresses.The past 3 decades has seen the number of virus species infecting chili as well as virus disease incidence considerably increase (Kenyon et al., 2014b). Likely, the most devastating chili-infecting viruses, especially in tropical and subtropical regions, are members of the whitefly-(*Bemisia tabaci*) transmitted *Begomovirus* (Geminiviridae), which cause chili leaf-curl disease (ChiLCD). In some of the hotspots for the disease, losses of 100% have been reported. Due to ChiLCD, farmers have switched from growing high value chili to low return cotton, maize, or other crops. In Madhya Pradesh, for example, the F_1 hybrid seed market was 15 tonnes prior 2014, but after the severe outbreak of resistance-breaking bipartite *Begomovirus*, the F_1 hybrid seed market was only 5 tonnes in 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. Furthermore, insecticides to manage the vector are often ineffective because they may only be applied once symptoms are seen by which time transmission of the virus has already occured (Kenyon et al., 2014a). An effective alternative to harmful insecticide applications for management of *Begomovirus* is the development of resistant cultivars (Shankarappa et al., 2008). However, there are several challenges to breeding chili cultivars resistant to *Begomovirus*.

In order to effectively breed for resistance, an understanding of the epidemiology, genetic diversity, and phylogeny of the pathogen is required. Despite the severe losses due to begomoviruses, systematic studies of the species of *Begomovirus* in different chili-production regions in Asia are limited. Kumar et al. (2015) conducted a survey across some of the major chili-growing regions in India. They found that ChiLCD was associated with a complex of begomoviruses, with a diverse group of betasatellites. The majority of the viruses were the result of intra-specific recombination. Furthermore, the betasatellites possessed high nucleotide variability and genetic recombination among them was also identified. The authors found that with a single exception, the *Begomovirus* species in India were monopartite. However, in the years since this study, more and more bipartite begomoviruses have been found in India and across Asia, and these are reported to cause greater suppression of host defense-related gene expression and breakdown of resistance (Singh et al. 2016; Dr. Anandalakshimi, personal communication). There is a need to conduct a new systematic *Begomovirus* survey in the major production regions of Asia to better understand the epidemiology, genetic diversity, and phylogeny of the newly developing chili-infecting bipartite *Begomovirus* species.

Sources of resistance to Begomovirus in pepper have been reported, including the *C*. *chinense* accessions BG-3821 in Mexico (Anaya-Lopez et al., 2003) and 'Bhut Jolokia' in India (Adluri et al., 2017). The *C*. *annuum* accessions 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., 2014a), GKC-29, BS-35, EC-497636 (Kumar et al., 2006), and 'Kalyanpur Chanchal' (Singh et al., 2016) have been reported to be resistant. Although several WorldVeg lines have been found to be resistant, their screening was done several years ago and the pathogen has likely changed; therefore, confirmation of resistance with recently emerged virus species is required.

Inheritance of resistance in BG3821 (to two New-World bipartite begomoviruses) appears to be controlled by two genes with duplicate recessive epistatic relation (Garcia-Neria and Rivera Bustamante, 2011). Similarity, for 'Bhut Jolokia', a single recessive gene has been reported to control resistance to *Pepper leaf curl virus* (PepLCV) (Rai et al., 2014). Preliminary results suggest that resistance in PBC 145 to PepYLCV is also recessive, but number of genes involved and gene action are not yet known (Prof. Suchila Techowongstein, personal communication). Recently, resistance in S-343 has been reported to be controlled by a single dominant gene (Thakur et al., 2019). However, the *Tomato leaf curl Joydepure virus* (ToLCJoV) implicated in their screening does not cause a particularly severe form of ChiLCD and the rating occurred at 90 days, which is quite early in the vegetative period. The modes of inheritance and gene action for other sources of resistance is limited. In tomato, it is known that a combination of dominant and recessive genes plays a role in resistance to *Begomovirus*.

Due to the largely recessive gene inheritance and the highly plastic genome of the pathogen, breeding for resistance to *Begomovirus* has been limited, despite a number of resistance sources identified. Another reason for the lack of resistant cultivars is the screening method used to identify resistance germplasm and evaluate segregating populations. Screening procedures include a biolistic procedure in which plasmid DNA is attached to tungsten microparticles and plant tissue is bombarded with pellets containing the microparticles (Garzón-Tiznado et al., 1993), grafting

inoculation method, where scions are grafted onto inoculated root stocks (Hernandez-Verdugo et al., 2001), and whitefly inoculation technique, where plants are infested with whiteflies known to be vectoring the virus and maintained throughout the season under screenhouse conditions.

There are numerous species of chili-infecting begomoviruses in Asia, and every year new species are identified. The tendency for genetic recombination, the acquisition of extra DNA components, and the synergistic interaction among different begomoviruses has resulted in the rapid emergence of new viruses that can infect new hosts, cause new disease symptoms, and overcome host resistance (Chakraborty et al., 2003; Singh et al., 2016; Varma and Malathi, 2003). In order to understand the basis for and to predict epidemic outbursts and global spread of *Begomovirus*, Jabłońska-Sabuka et al. (2015) utilized a mathematical model. They found that intensive farming and breeding partially resistant cultivars were the major triggers for aggressive virus adaptability through mutation speed-up. Therefore, it would be beneficial to use an integrated pest management (IPM) approach that combines resistant cultivars (potentially multiline cultivars) with different whitefly and viral control measures. It has been estimated that farmers that adopt low, medium, and high integrated management strategies for *Begomovirus* could improve incomes by 17, 26, and 80%, respectively (Swaminathan et al., 2016).

A core collection represents the genetic diversity of a given crop with minimal redundancy. The core collection allows for an existing collection to be more accessible through designating a small group of accessions that would be the focus of evaluation and use and would provide an entry point to the larger collection that it aims to represent. As part of the EU Horizon 2020 (H2020) funded project From Genotype to Phenotype-Solanaceae (G2P-Sol), WorldVeg and our collaborators have developed a core collection of *Capsicum*, consisting of 400 accessions based on sequence based genotyping. In order for this core collection to reach its maximum potential, extensive phenotyping for traits of strategic importance is required. The benefits of phenotyping the core collection of *Capsicum* against ChiLCD in the hotspots for the disease include: 1) providing the opportunity to identify novel sources of resistance as the core collection represents the total genetic diversity of the crop, 2) rapid identification of resistance-associated loci, because the collection is already genotyped, and 3) allow for targeted accession selection for future resistance screening based on relatedness, passport data, and other unique characteristics.

Proposed Methods/Activities

This project will take place over a 3 year period

Activity 1. Multi-location trials of WorldVeg resistant chili lines in selected hot spots in Asia.

A set of 10 WorldVeg developed advanced pepper breeding lines that have been previously identified as being resistant to different members of *Begomovirus* in India and recently in Thailand using a whitefly inoculation technique will be distributed to APSA Consortium Project partners. Sufficient seed of these lines will have been cleared by BAPHIQ for distribution out of Taiwan and be available in the first year (2020) to establish at least 15 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 begomoviruses are prevalent in chili. Final choice of which sites will be used in each year of the project will depend on what sites are offered by project supporting APSA member companies and will be discussed during the 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 in 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 ChiLCD lines or hybrids. Each trial will be grown over the local peak season for ChiLCD

pressure. 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*.). On a regular basis (every 30 days) the plants in each trial will be scored for ChiLCD incidence and 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. 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 ChiLCD 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. Multiplication of the H2020-G2P Sol Core Collection of *Capsicum* germplasm accessions in isolation.

Through genotyping by sequencing, the H2020 G2P-Sol project developed a core collection of *Capsicum* consisting of 400 accessions, which represents the total genetic diversity of the crop. In order for the core collection to be available for distribution, the lines must be multiplied in isolation to prevent cross-pollination, the seed will be cleaned, tested by WorldVeg Seed Health and Quarantine (SHQ) unit, and cleared by the Taiwan Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ). Therefore, following the WorldVeg developed best management practices, the core collection of *Capsicum* will be multiplied at WorldVeg HQ, Taiwan. Fine-mesh row covers will be used to exclude insect pollinators, greatly reducing the risk of cross-pollination. Seed will be multiplied during the fall/winter season to ensure high-quality seed is produced and germination rate will remain high through short- and long-term storage and shipping. All seed will be thoroughly cleaned to remove fruit or plant debris and any broken or discolored seeds prior to treating with HCl for 15 min and TSP for 1 h. Then using PCR, presence of Solanaceae-infecting Pospiviroids will be detected following previously published methods at SHQ. The core collection of *Capsicum* will then be sent to BAPHIQ for phytosanitary (including viroid) testing and certification.

By August of 2019, the first 100 lines of the G2P-Sol Core Collection of *Capsicum* will be multiplied and cleared through BAPHIQ. These first 100 accessions will be distributed to member companies along with Activity 1 lines for the first round of testing. The remaining 300 core collection accessions will be distributed according to the timeline listed below.

Activity 3. Characterization of H202-G2P-Sol Core Collection of *Capsicum* for ChiLCD and other abiotic and biotic stress resistance.

High quality seeds of the newly multiplied 400 lines within the core collection of *Capscium* will be provided by WorldVeg HQ to member companies (~1.0 g seed/accession, depending on availability) and it is anticipated that there will be sufficient seed to establish at least 15 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 begomoviruses are prevalent in chili. Final choice of sites will be done as in Activity 1. Management, trial evaluation and field visits will be as outlined for Activity 1. If a participating APSA member company has facilities for faster throughput screening, such as net screenhouses with virus source plants and big population of whiteflies viruliferous with a severe local ChiLCD strain, then these could be used instead of the open field trial.

Activity 4. Collection and molecular characterization of chili-infecting *Begomovirus* species in Asia.

Samples of chili lines showing symptoms of virus infection (from very mild to severe) from each field trial (set up in activity 1a above) will be collected and dried using the protocol provided by WorldVeg and then sent to WorldVeg HQ or the WorldVeg South Asia Regional Center in Hyderabad, if the trial is in India (Staff from WorldVeg HQ will travel to the WorldVeg South Asia Center to guide and assist in the analysis there as Indian government legislation prevents the molecular diagnosis and identification being done outside India unless there is collaboration with and approval from an ICAR agency). Each sample will be subject to Polymerase chain reaction (PCR) with universal primers for Begomovirus DNA-A, DNA-B, and alpha and betasatellites. Sequencing will be used to identify which species of *Begomovirus* is present in selected PCR-positive samples. This is to determine which ChilCD species are prevalent at each trial location. (Depending on availability of additional resources, a strategy of rolling-circle amplification of begomovirus and satellite DNAs, bar-coding and high-throughput sequencing (Oxford Nanopore MinION) could be used to obtain the full-length sequences of the complete circular DNA virome from more samples from the test sites). As well as the samples from the trials, APSA member companies will be encouraged to provide additional samples along with GPS coordinates from chilli with typical ChiLCD symptoms from other locations. A maximum of 4 additional samples per member company may be provided for analysis.

Deliverables

1. Historically resistant WorldVeg chili breeding lines evaluated for resistance against newly evolved/recombined ChiLCD isolates.
2. Increased understanding of G x E effects on resistance to the most serious viral disease affecting chilli production in Asia.
3. Potentially novel sources of resistance to ChiLCD identified from the core collection of *Capsicum* which has not been systematically accessed and screened by public or private breeding programs previously.
4. Improved understanding of the phylogenetic structure of the ChiLCD pathogen and the role genetic recombination plays in the breakdown of resistance to ChiLCD in the major production regions in Asia.

Timeline of activities

	Year 1		Year 2		Year 3	
	1st half	2nd half	1st half	2nd half	1st half	2nd half
Distribution of previously identified WorldVeg resistant lines as well as the first 100 lines of the core collection to member companies for multi-location trialing.						
Multiplication of the G2P-Sol core collection of *Capsicum*						
Seed extraction and cleaning of multiplied seed						
Testing through WorldVeg SHQ and submission to BAPHIQ for phytosanitary testing and certification						
Distribution of the remaining 300 G2P-Sol Core Collection of *Capsicum* lines to member companies						
Multi-location trials of G2P-Sol Core Collection of *Capsicum* in the hotspots for ChiLCD						
Collection and molecular characterization of chili-infecting *Begomovirus* species in Asia						
Results compiled, final report developed and disseminated to all member companies						

References

Adluri, P.K., G.M. Baldoldiya, and P.D. Nath. 2017. Screening of Bhut Jolokia (*Capsicum chinense* Jacq.) germplasm of North East India against chili leaf curl virus. Intl. J. Pure Appl. Bioscience 5:1189-1196.

Anaya-Lopez, J.L., I. Torres-Pacheco, M. Gonzalez-Chavira, J.A. Garzon-Tiznado, J.L. Pons-Hernandez, R.G. Guevara-Gonzalez, et al. 2003. Resistance to geminivirus mixed infections in Mexican wild peppers. Hortscience 38:251-255.

Borah, B.K. and I. Dasgupta. 2012. Begomovirus research in India: a critical appraisal and the way ahead. J. Biosci. 37:791-806.

Bosland, P.W. and E.J. Votava. 2012. Peppers: vegetable and spice capsicums. 2nd ed. CAB International, Oxfordshire, U.K.

Chakraborty, S., P.K. Pandey, M.K. Banerjee, G. Kalloo, and C.M. Fauquet. 2003. Tomato leaf curl Gujarat virus: a new *Begomovirus* species causing severe leaf curl disease of tomato in Varanasi, India. Phytopathology 93:1485-1495.

DeWitt, D. and P.W. Bosland. 1993. The Pepper Garden. Ten Speed Press, Berkeley, CA.

Fu, Y.B., M.H. Yang, F. Zeng, and B. Biligetu. 2017. Searching for an accurate marker-based prediction of an individual quantitative trait in molecular plant breeding. Frontiers Plant Sci. doi:10.3389/fpls.2017.01182

Garcia-Neria, M.A., and R.F. Rivera-Bustamante. 2011. Characterization of *Geminivirus* resistance in an accession of *Capsicum chinense* Jacq. Mol. Plant-Microbe Interact. 24:172-182.

Garzón-Tiznado, J.A., I. Torres-Pacheco, J.T. Ascencio-Ibañez, L. Herrera-Estrella and R.F. Rivera-Bustamante. 1993. Inoculation of peppers with infection clones of a new geminivirus by biolistic procedure. Phytopathology 33: 514–521.

Hernandez-Verdugo, S., R.G. Guevara-Gonzalez, R.F. Rivera-Bustamante, and K. Oyama. 2001. Screening wild plants of *Capsicum annuum* for resistance to pepper huasteco virus (PHV): Presence of viral DNA and differentiation among populations. Euphytica 122: 31–36.

Jabłońska-Sabuka, M., R. Kalaria, and T. Kauranne. 2015. A dynamical model for epidemic outbursts by *Begomovirus* population clusters. Ecol. Modeling 297:60-68.

Kahane, R., T. Hodgkin, H. Jaenicke, C. Hoogendoorn, M. Hermann, J.D.H. Keatinge, J. d'Arros Hughes, S. Padulosi, and N. Looney. 2013. Agrobiodiversity for food security, health, and income. Agr. Sustainable Development 4:671-693.

Kenyon, L., S. Kumar, W,S. Tsai, and J. d'A. Hughes. 2014a. Virus diseases of peppers (*Capsicum* spp.) and their control. In: G. Loebenstein and N. Katis (eds.). Advances in virus research. Vol. 90. Academic Press p. 297-254.

Kenyon, L., W.S. Tsai, S.L. Shih, and L.M. Lee. 2014b. Emergence and diversity of begomoviruses infected solanaceous crops in East and Southeast Asia. Virus Res. 168:104-113. doi: 10.1016/j.virusres.2013.12.026.

Kumar, R.V., A.K. Singh, A.K. Singh, T. Yadav, S. Basu, N. Kushwaha, B. Chattopadhyay, and S. Chakraborty. 2015. Complexity of begomovirus and betasatellite population associated with chilli leaf curl disease in India. J. General Virol. 96:3143-3158.

Kumar, S., S. Kumar, M. Singh, A.K. Singh, and M. Rai. 2006. Identification of host plant resistance to pepper leaf curl virus in chilli (*Capsicum* species). Sci. Hort. 110:359-361.

Montes, H. 2010. Compilation and analysis of existing information on the species of the genus *Capsicum* grown and cultivated in Mexico. Final report. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experi-mental Bajío, Celaya, Gto. México.

Rai, V.P., R. Kumar, S.P. Singh, S. Kumar, S. Kumar, M. Singh, and M. Rai. 2014. Monogenic recessive resistance to *Pepper leaf curl virus* in an interspecific cross of *Capsicum*. Sci. Hort. 172:34-38.

Shankarappa, K., S. Sriharsha, K.T. Rangaswamy, D.S. Aswathanarayana, H.A. Prameela, R.S. Kulkarni, V. Muniyappa, A.M. Rao, and M.N. Marauthi. 2008. Development of tomato hybrids to tomato leaf curl virus disease in South India. Euphytica 164:531-539.

Sherman, P.W. and J. Billing. 1999. Darwinian gastronomy: Why we use spices taste good because they are good for us. Bioscience 49:453-463.

Singh, A.K., N. Kushwaha, and S. Chakraborty. 2016. Synergistic interaction among begomoviruses leads to suppression of host defense-related gene expression and breakdown of resistance in chilli. Appl. Microbiol. Biotech. doi:10.1007/s00253-015-7279-5.

Smith, C.E. 1967. Plant remains. p. 220-225. In: D.S. Byes (ed.). The prehistoric history of the Tehuacan Valley vol. 1. Environment and subsistence. University of Texas Press, Austin, TX.

Smith, S.H. 2015. In the shadow of a pepper-centric historiography: understanding the global diffusion of Capsicums in the sixteenth and seventeenth centuries. J. Ethnopharmacol. 167:64-77.

Srivastava, A., M. Mangal, R.K. Saritha, and P. Kalia. 2017. Screening of chilli pepper (*Capscium*spp.) lines for resistance to the begomovirus causing chili leaf curl disease in India. Crop Protection 100:177-185.

Srivastava, A., M. Mangal, R.K. Saritha, L.J. Santosh, G. Uttamgir Gosavy, and P. Kalia. 2015. Natural epiphytotic screening of chilli germplasm lines against leaf curl virus complex. Intl. J. Tropical Ag. 33:3581-3586.

Swaminathan, B., K.C. Siva Balan, N. Anadaraja, N. Manikanda Boopathi, P. Schreinemachers, R. Srinivasan, and M.H. Wu. 2016. Profitability of begomovirus management strategies among chilli farmers in Tamil Nadu: a gross margin impact analysis. Indian J. Ag. Res. 50:159-166.

Thakur, H., S.K. Jindal, A. Sharma, and M.S. Dhaliwal. 2019. A monogenic dominant resistance for leaf curl virus disease in chilli pepper (*Capsicum annuum* L.). Crop Protection 116:115-120.

Varma, A. and V.G. Malathi. 2003. Emerging geminivirus problems: a serious threat to crop production. Ann. App. Biol. 142:145-164.

Wall, M.M. and P.W. Bosland. 1998. Analytical methods for color and pungency of chiles (*Capsicums*). p. 347-373. In: D. Wetzel and G. Charalambous (eds.). Instrumental methods in food and beverage analysis. Elsevier B.V., Amsterdam, Neth.

Weinberger, K. and T.A. Lumpkin. 2007. Diversification into horticulture and poverty reduction: A research agenda. World Develop. 35:1464-1480.