Heat stress tolerance of tomato & pepper

Research Proposal

Prepared for

The Asia and Pacific Seed Association (APSA)

Under the

APSA-WorldVeg Vegetable Breeding Consortium

By

World Vegetable Center

Proposal Summary

Project title	Heat stress tolerance of tomato & pepper	
Submitted to	APSA	
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Project duration	3 years (July 2021-June 2024)	
Funding requested (US$)	350,000	Minimum of 15 companies with US$23,333 per company
		Maximum of 25 companies with US$14,000 per company

Objective

The overall objective is to strengthen heat stress tolerance breeding by identifying new sources of heat stress tolerance and characterizing the major component traits associated with heat tolerance for pepper and tomato.

Background

Harnessing crop tolerance to elevated temperatures is essential for sustaining vegetable production in tropical areas and will become even more important under climate change scenarios. Pollen is the most heat susceptible stage in many crop species (Hedhly et al., 2009, Giorno et al., 2013) and without viable pollen, fruit set of tomato, pepper and other fruit vegetables is reduced or completely impeded. Selection for heat tolerant plants can be done by monitoring fruit set, but this method does not discriminate between the various flower traits involved (pollen viability, pollen germination, style exertion, stigma non-receptivity, arrested pollen tube growth, or other traits). Furthermore, it requires measuring fruit set over an extended time and is sensitive to variations in temperature that could permit an otherwise heat sensitive plant to set fruit. Pollen viability is usually monitored by staining the harvested pollen and counting pollen with specific staining patterns under a microscope (Heslop-Harrison et al., 1984). This is a laborious and time consuming technique with limited throughput. Also, short periods of lower temperatures can allow otherwise sensitive plants to produce viable pollen. Such erroneous results cannot be easily corrected, as the low throughput of the method does not allow measurement of large numbers of samples required to phenotype large breeding populations.

For crops like tomato and pepper, pollen viability alone does not sufficiently inform about the capacity of pollen to fertilize female germ cells. Instead pollen germination data are required to estimate heat tolerance of pepper pollen (Mercado et al., 1994). In vitro pollen germination may be affected by genotype specific differences in compatibility with germination media. Like pollen viability tests, this method is laborious and has very low throughput, limiting its usefulness in breeding. Therefore, WorldVeg has recently started to characterize WorldVeg pepper and tomato heat tolerant sources and breeding lines for pollen number and pollen activity using impedance flow cytometry (IFC) (Heidmann et al., 2016). IFC is an efficient, label-free and reliable technique to analyze pollen activity in a species-independent manner in high throughput mode. Active pollen is defined as viable pollen that will likely germinate, and in pepper we found that pollen activity using IFC has a significant positive correlation with low throughput pollen viability evaluated by staining ($r = 0.75$). Preliminary results indicate that the average pollen number per flower is associated with tolerance to high temperature stress, although these results need to be confirmed.

Dry and humid heat may affect crops in a different manner, especially in tomato where different reactions to dry and humid heat have been observed. Therefore, heat stress tolerance screening is best complemented by multilocation field trials to test the response to various kinds of heat. Heat stress tolerant pepper and tomato breeding lines have been developed by WorldVeg. Recently, the Horizon 2020 G2P-Sol program (g2p-sol.eu) has provided global tomato and pepper core collections that are currently being screened for heat stress tolerance, resulting in best bet sets of heat tolerant tomato and pepper accessions for multi-location testing. Sweet pepper is more heat sensitive than chili pepper, therefore improvement of heat tolerance will likely have a greater impact on sweet than on chili pepper. For tomato, the Horizon 2020 TomGem project (tomgem.eu) made available additional heat stress tolerant sources and segregating populations. Materials with high tolerance will be selected from these germplasm panels and investigated under field conditions for their performance under dry and humid heat stress to obtain tolerant materials for heat stress tolerance breeding.

While the use of multilocation trials to determine the role different environments play in heat tolerance in tomato and pepper is critically important, there are certainly limitations to this approach. Across South and Southeast Asia, pests and diseases are major limiting factors for tomato and pepper production, most notably are members of the genus *Begomovirus*. Furthermore, the pesticides used to manage pests and diseases can contain plant growth regulators, which can influence the results of screening for abiotic stress tolerance in field settings (Dr. Seetharam Annadana, personal communication). In order to better access the performance of the heat tolerant lines and to more accurately parse out the individual or combination of traits (pollen viability, pollen germination, style exertion, stigma non-receptivity, arrested pollen tube growth, fruit set, photosynthetic capacity, among other traits) contributing to heat tolerance, in-depth characterization of the lines under controlled conditions is required. A phytotron is an enclosed research greenhouse used for studying interactions between plants and the environment. A phytotron allows users to grow plants under highly controlled conditions (temperature [±1°C], humidity, and light intensity [±10 nm]), limiting or eliminating confounding factors such as pests and diseases as well as the PGRs in some pesticides, and monitor the plant's performance during its lifecycle. Through the Research Infrastructure Modernization

project (worldveg.org/RIM), funded by the Government of Taiwan/Council of Agricultural, WorldVeg plans to establish a phytotron platform at our Center in Taiwan.

Proposed Methods/Activities

Activity 1. Multilocation trials of WorldVeg tomato and pepper sources of heat tolerance

A set of 15-20 tomato heat tolerance sources (including parents of RIL populations) and 15-20 chili heat tolerance sources will be provided to project member companies for multilocation trials within the first 6 months of the project.

Prior to distribution, the lines will be multiplied, the seed will be cleaned, tested by the WorldVeg Seed Health and Quarantine (SHQ) lab, and cleared by the Taiwan Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ). Therefore, following the WorldVeg developed best management practices, the lines 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 in pepper. 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 lines will then be sent to BAPHIQ for phytosanitary (including viroid) testing and certification. The required Genebank fees associated with seed release will be covered by the project budget, but each individual company will be responsible for covering the costs of seed shipment, due to the diversity in locations of the member companies in the project.

Sufficient seed of these lines will be cleared by BAPHIQ for distribution out of Taiwan and be available in the first year (2021-22) to establish up to 25 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 by begomoviruses are minimal, but where there are sufficiently high temperatures to assess heat tolerance. 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 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 their own heat tolerant lines or hybrids. Each trial will be grown over the local peak season for high temperature stress. 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 key data collected by each company will be finalized in the inception workshop but will include indicators of heat tolerance such as yield, pollen staining, and fruit set measure by tagging flowers and determining how many set to form fruits in field conditions. 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 the trial is well established in order to observe the performance of the lines and make independent assessment of the heat tolerance 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.

Control trials will be conducted at WorldVeg HQ in Taiwan under hot and humid conditions, where we will also evaluate the pollen traits of the lines using impedance flow cytometry following the protocol established by Lin et al. (2020) and traditional pollen viability testing using staining and microscopy in addition to the other heat tolerance traits such as stigma exertion, fruit set, among others.

Activity 2. Multilocation trials of advanced breeding and finished lines for heat tolerance

Based on the performance of the trials in the first year of the project, a subset of sites will be selected to conduct a second trial. For the second round of trials, companies will receive tomato recombinant inbred lines (RILs) combining heat tolerance and *Ty* genes and/or high performing heat tolerant sweet pepper finished lines for greenhouse trials (no ChiLCD resistance).

WorldVeg has developed four tomato biparental RIL populations designed to map heat tolerance in tomato. Heat tolerant parents of these populations include CLN1621L, Siberia, Tomato337 (from Nigeria), and Divisoria-2 (Philippines). The heat sensitive parent of the latter three populations is Tstar-29-4-3-12-6-11-4 (Ty-1 inbred line selfed from the hybrid 'Tovi Star'). CA4 is the heat sensitive parent crossed to CLN1621L. All RILs of the CLN1621L x CA4 population are homozygous for Ty-3, a big advantage for open field trials where TYLCD is a problem. *Ty-1* was not fixed in the other three mapping populations. A subset of the Tstar-Siberia RILs was evaluated in the Taiwan 2020 humid summer season for pollen viability, stigma exception, and fruit set and QTL analysis of heat-related traits is ongoing at National Taiwan University. Based on the QTL analysis, a set of Tstar-Siberia RILs will be selected for multilocation testing. The CLN1621L x CA4 and possibly the other two RIL populations will be phenotyped for heat traits in Taiwan and subsets selected for multiplication testing.

Several heat tolerant sweet pepper finished lines have been developed and released by WorldVeg in Taiwan. Recently, two sweet pepper hybrids have been released in Taiwan with a high level of heat tolerance in collaboration with Taichung District Agricultural Research and Extension Station. Through multilocation trials in farmer greenhouses and at WorldVeg HQ, we found the new hybrids have yields that are not different from commercial hybrids, but the level of heat tolerance, as determined by fruit number, fruit size, pollen activity, and nutritional content was higher. The heat tolerant inbred sweet pepper lines have not been trialed outside of Taiwan, and the seed will need to be tested by SHQ and BAPHIQ prior to distribution. Through this project, inbred sweet pepper lines with heat tolerance will be evaluated by member companies in multilocation trials in protected cultivation (to present ChiLCD) following WorldVeg provided protocols.

Activity 3. Advanced phenotyping of heat tolerant lines

A selected subset of tomato and chili heat tolerant sources, based on the trials conducted in the first year of the project, along with heat sensitive checks will be used for advanced phenotyping in Taiwan. The trials will be conducted in Phytotrons and climate-controlled greenhouses at WorldVeg HQ. The purpose of conducting these experiments in controlled environment conditions is to parse out the different components of heat tolerance while eliminating the confounding factors of fluctuating environment and biotic stresses. Experiments will be carried out following a complete randomized design, with plant number (biological replication) depending upon accessions selected and space available, but we will maximize replication. The environmental conditions for the chili heat tolerance experiment will be 28-30°C and 38-40°C night and day temperatures with a 12-hour photoperiod. Relative humidity is difficult to accurately control in phytotrons or growth chambers, but we will aim for low humidity of around 40%. Tomato sources will be subjected to maximum/minimum temperatures of 35°C and 25°C, respectively.

Extensive phenotyping will be done during the controlled environment experiment, including evaluation of all pollen traits (pollen activity, pollen concentration, pollen viability, pollen germination, etc.) and other floral traits (antheridial cone splitting, anther dehiscence and pollen release, stigma exertion, flower number, fruit set). We will also conduct morphological and physiological analysis which may or may not include chlorophyll fluorescence and content (SPAD), stomatal conductance, leaf temperature, plant height, canopy width, shoot dry mass, root dry mass, root-shoot ratio. The data collected in these experiments will be compared to the performance of the heat tolerant sources in the multilocation trials across Asia. The overall goal is to determine which traits are most associated with yield and performance of the lines in open field conditions. This will not only enable us to improve selection efficiency in breeding, but also provide more accurate targets in conducting QTL/gene identification and molecular marker development to support marker assisted selection for heat tolerance.

Deliverables

1. Sources of heat tolerance in tomato and pepper under humid and dry conditions validated across Asia.
2. Correlation between pollen activity, pollen number and fruit set determined for more efficient selection.
3. Association between individual heat tolerance traits (measured in controlled environmental conditions) and performance of lines in multilocation trials across Asia for improved selection efficiency and a basis for future gene discovery and marker assisted selection.
4. Tomato and pepper lines (15-20) to serve as sources of high pollen number, high pollen activity, and heat tolerance.
5. Horticulturally adapted and consumer accepted red and yellow heat tolerant sweet pepper (capsicums, bell pepper), chili pepper and tomato inbred lines.

Duration and Budget

This project will be conducted from 1 July 2021 to 30 June 2024, with the following budget:

Budget item	Cost (US$)
Personnel	87,504
Supplies & operations	205,383
Sub-total	292,887
Indirect costs (19.5%)	57,113
Total	350,000

Timeline of activities

Activity / Year & month	Year 1		Year 2		Year 3	
	2021	2022	2022	2023	2023	2024
	Jul-Dec	Jan-Jun	Jul-Dec	Jan-Jun	Jul-Dec	Jan-Jun
Inception workshop, experimental design, site selection, data collection finalized						
Multiplication, cleaning, testing, certification & distribution of seed						
Multilocation trials of tomato and chili heat tolerant sources across Asia						
Data analysis, site selection, and distribution of tomato RILs and finished sweet pepper lines						
Multilocation trials of tomato RILs and finished sweet pepper heat tolerant lines across Asia						
Midterm workshop to assess the trials and make changes in experimental plan as needed						
Advanced phenotyping of selected tomato and chili heat tolerance sources in controlled environment conditions						
Final data collection, compilation and analysis						
Final workshop, data staring, and planning of next steps in heat tolerance breeding						

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