



**AVRDC**

**The World Vegetable Center**

## **Final Report**

**(April 1, 2005 – August 31, 2008)**

**Development of Locally Adapted, Multiple Disease-Resistant and High Yielding Chili (*Capsicum annuum*) Cultivars for China, India, Indonesia and Thailand - Phase II**

International Agricultural Research  
Project No. 03.7860.4 – 001.00  
Contract No. 81077027

Submitted by  
**AVRDC – The World Vegetable Center**  
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to  
**Deutsche Gesellschaft für Technische Zusammenarbeit**  
**(GTZ) GmbH**

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- Annex 1: Protocol for conducting inheritance study on resistance to bacterial wilt in chili peppers
- Annex 2: Multi-disease resistant progenies selected
- Annex 3: Inheritance study

1. **Name of IARC:**  
AVRDC - The World Vegetable Center
2. **Project title:**  
Development of locally adapted, multiple disease-resistant, high yielding chili (*Capsicum annuum*) cultivars for China, India, Indonesia and Thailand – Phase II
3. **GTZ Project number and Contract number:**  
Project No. 03.7860.4-001.00  
Contract No. 81077027
4. **Reporting period:**  
1 April 2005 to 31 August 2008
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6. **Collaborating institutions and staff including NARS and German partners**  
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7. **Project description**  
**Goal:** Contribute to increased income and reduced risk of resource poor Asian farmers engaged in chili production  
**Purpose:** Farmers cultivate multi-disease-resistant chili varieties in an economical and environmentally sustainable manner

## Outputs:

1. Resistance sources to new pathogens (Phytophthora and Chili leaf curl disease) identified at AVRDC and confirmed in NARS
2. Efficient molecular diagnostics and screening methodologies developed for all pathogens
3. Molecular markers for targeted diseases developed or refined for use
4. Mode of inheritance of resistance to each of the pathogens determined by classical methods complemented by molecular markers and disease diagnostics
5. Selection of multi-disease resistant chili progenies completed and advanced
6. Gene constructs developed that provide resistance to aggressive strains of *Cucumber mosaic virus* (CMV) in Thailand and China and of *Chilli vein mottle virus* (ChiVMV) in China (University of Hamburg)

## 8. Major Research findings

An overview of the progress of the five outputs as outlined in the original Project Workplan is presented below:

### OUTPUT 1: Resistance sources to new pathogens (Phytophthora and Chili Leaf Curl Disease) identified at AVRDC and confirmed in NARS

#### Activity 1.1 Isolates of *Phytophthora* and Chili leaf curl disease collected and characterized

##### Phytophthora

AVRDC: 11 pathotype 1, 49 pathotype 2 and 71 pathotype 3 isolates of *Phytophthora capsici* were characterized.

China: 42 isolates collected including 5, 16 and 4 isolates were identified as pathotype 1, 2, and 3, respectively of *P. capsici*. Pathotype 2 is the predominant one.

India: 70 isolates of pepper-infecting phytophthora were collected from Bangalore, Hassan, Raichur, Belgaum and Gulbarga District in Karnataka State. Two distinct groups (based on sporangial morphology) were identified. 16, 6, and 11 isolates were identified as pathotype 1, 2 and 3, respectively.

Indonesia: All 6 isolates collected were identified as pathotype 1.

Thailand: 12 isolates were collected from 4 chili production areas in different provinces, and identified as pathotype 3

##### Chili leaf curl disease

India: Eighteen begomovirus isolates from across India (Andhra Pradesh, Haryana, Rajasthan, Tamil Nadu, Uttar Pradesh and W. Bengal) have been sequenced. By sequence comparisons seven distinct begomoviruses were identified including *Chilli leaf curl virus* (ChiLCV; CHL81 from Tamil Nadu), *Tomato leaf curl Sri Lanka virus* (ToLCSLV; CHL24 from Tamil Nadu), *Pepper leaf curl Bangladesh virus* (PepLCBDV; WCH4 from West Bengal), *Pepper leaf curl Pakistan virus* (PepLCPKV; CLN148 from Andhra Pradesh), *Tomato leaf curl New Delhi virus* (ToLCNDV; CLN189 from Haryana and CLN206 from Rajasthan) and two new viruses (CHL1 from Uttar Pradesh and CHL2 from Rajasthan).

Thailand: Three begomovirus isolates (PCB, SSK1, SSK2) have been isolated and maintained. They infect okra, tomato, eggplant and some cucurbit species. Two (PCB, SSK2) isolates are closely related to *Tobacco curly shoot virus* (TbCSV), the other isolate seems to be a new virus.

Indonesia: *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) isolate from West Java has been sequenced.

China: Based on a total of 370 samples collected so far from Hainan Island, Guilin, Guangxi and Xian, pepper-infecting begomoviruses were not detected in China.

## **Activity 1.2 Screening of selected AVRDC germplasm for Phytophthora (Pc) and Chili leaf curl disease resistance**

### **Phytophthora**

Screening by soil drench of *Pc* zoospore suspension ( $10^5$  zoospores/ml) with 5ml per plant at 35 days old seedlings under controlled environmental condition ( $>25^\circ\text{C}$ ) was found to give reliable results at AVRDC. 6 (CM331, CM334, PI189550, PI201232, PI201234, PI201238) out of 4,000 *Capsicum annum* accessions were identified as highly resistant to *P. capsici* (pathotype 3) at AVRDC and distributed to the 4 NARS in August 2005.

China: CM331, CM334, PI189550, PI201232, PI201234 and PI201238 were identified as having durable resistance to pathotype 2.

India: CM331, CM334, PI201232, PI201234 and PI201238 were identified as having durable resistance to the three pathotypes. PI189550 was only resistance to pathotype 2 and CNPH703 was resistant to pathotype 1 alone.

Indonesia: CM331, CM334, PI201232, PI201234, PI201238 and PI2189550 were identified durable resistance to pathotype 1.

Thailand: CM331, CM334, PI189550, PI201232, PI201234 and PI201238 exhibited durable resistance to local pathotype 3.

### **Chili leaf curl disease**

A seedling screening methodology has been developed at AVRDC. The seedlings were screened in a small 50-mesh screenhouse for LCV resistance using viruliferous *Bemisia tabaci* biotype B. The methodology has been distributed for germplasm screening by NARS. A total of 155 pepper lines including some with leaf curl disease resistance and from the AVRDC collections have been assembled and multiplied by AVRDC, and have been sent to India, Thailand and Indonesia for testing the resistance with local leaf curl virus isolates.

India: Resistance screening has been conducted by field trial. Fifteen lines (line nos. 12, 27, 30, 41-3, 43, 46, 47, 57, 59, 73, 82, 86, 90-3, 92-3 and 100-2) were identified in Tamil Nadu that were highly resistant with  $<10\%$  virus incidence and 28 lines were resistance (10-20% virus incidence). In Haryana, five (line nos. 33, 35, 45, 59 and 86) out of 34 lines were identified as being highly resistant ( $<10\%$  virus incidence) and eight were resistant (10-24.5% virus incidence).

Indonesia: All locally available commercial cultivars were susceptible to leaf curl virus disease. Five (PBC 495, ICPN 12#4, ICPN 7#3, VC 211 and VC246) out of 20 AVRDC lines were found to be highly resistant ( $<10\%$  virus incidence) to local virus isolate. Some of these lines also carry resistant to *Chilli veinal mottle virus* (ChiVMV; Potyvirus), Potato virus Y (PVY), *Cucumber mosaic virus* (CMV) and/or bacterial wilt.

Thailand: Seven (PY6, 7, 8, 128, 129, 130 and 131) out of 156 lines were found to be highly resistant ( $<10\%$  virus incidence) to SSK1, the apparently new begomovirus species.

## **OUTPUT 2: Efficient molecular diagnostics and screening methodologies developed for all pathogens**

### **Activity 2.1 Development and testing of improved diagnostics for single and multiple pathogens**

#### **2.1.a PCR-based diagnostics for 3 species of Anthracnose, Phytophthora, Bacterial Wilt, CMV, ChiVMV, and begomoviruses causing Chili leaf curl disease**

##### **Anthracnose**

A PCR-based protocol for distinguishing the 3 *Colletotrichum* species (*C. acutatum* (Ca), *C. capsici* (Cc) and *C. gloeosporioides* (Cg) was distributed to all NARS. A one-day hands-on training was conducted by the AVRDC Mycologist for the NARS Mycologists at Hunan Plant

Protection Institute, Hunan AAS, China following the Project Planning Workshop. The following species were identified using PCR:

**AVRDC:** Restriction Fragment Length Polymorphism of rDNA Internal Transcribed Spacer was used to identify *Colletotrichum* species infecting chili in Taiwan and the new species *C. boninense* was identified. This is the first report of *C. boninense* causing chili anthracnose. Three hundred and three *Ca* isolates, 41 *C. boninense* (*Cb*) isolates, 61 *Cc* isolates and 97 *Cg* isolates were identified.

**China:** Six *Ca* isolates, 5 *Cc* isolates and 71 *Cg* isolates were identified. This is the first report of *Ca* causing anthracnose of chili in China.

**India:** Two *Ca* isolate, 53 *Cc* isolates and 38 *Cg* isolates were identified. This is the first report of *Ca* based on phenotypic characterization.

**Indonesia:** Seven previous *C. gloeosporioides* isolates were identified as *C. acutatum* using specific primer CaINT2. A total of 54 isolates (93.1%) and 4 isolates (6.9%) were identified as *C. acutatum* and *C. gloeosporioides*, respectively. *C. acutatum* is the first report in Indonesia and it is the predominant one.

**Thailand:** Sixty nine isolates of *Colletotrichum* spp. were characterized by PCR and three species *C. acutatum*, *C. capsici* and *C. gloeosporioides* were identified. This is the first report of *C. acutatum* causing anthracnose of chili in Thailand.

### Phytophthora

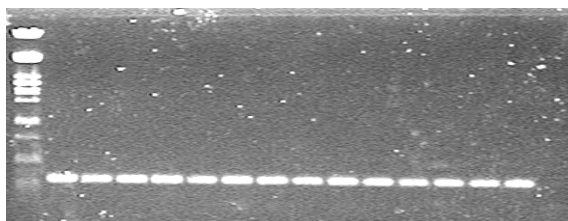
Species-specific primers CapFW/Cap RV2 amplifying a region of 595bp length was developed for *Pc* identification with other Phytophthora species. a race-specific DNA marker was developed for rapid identification. AFLP technology was established (selective amplification primer: EcoAC/Msec CT) for analyzing genetic variation of *P. capsici* at AVRDC.

### Bacterial Wilt

Isolation of *Ralstonia solanacearum* is normally done using a semi-selective medium named TTC. However, colonies of the pathogen on TTC plates were confused with other saprophytic bacteria. A species-specific primer pair (AU759/760) has been developed by Opina *et al* (1997). During the planning workshop of Phase 2, NARS of China, India, Indonesia and Thailand were trained for the detection of *R. solanacearum* by PCR. Later on, the primers and control DNA were provided by AVRDC for to each NARS.

**China:** A total of 20 isolates collected from chili pepper plants in the disease nursery of Hunan Vegetable Research Institute were confirmed to be *R. solanacearum* following the boiled cell protocol (Fig. 1).

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



**Figure 1** PCR-based diagnostics of bacterial wilt in China. Lane 1: Lambda DNA/PstI marker; Lane 2 to 14: Strain Vc58a-8-1-1 (19), VcB58a-8-1-1 (18), CCA5731 (17), P12012-34 (16), CCA5781 (15), PBC137 (14), Vc246 (13), Vc246 (13), PI2012-34 (16), CCA5409 (12), ZCPN12-4 (11), PBC602 (10), CCA5731 (17); Lane 15: positive control (Pss71); Lane 16: negative control

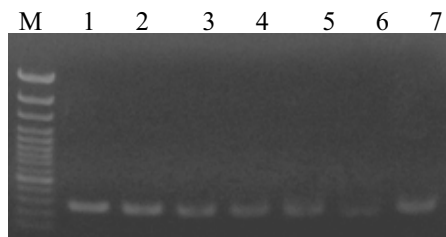
**India:** Using the AVRDC protocol, 20 strains isolated from wilted tomato plants grown in bacterial wilt disease nursery at Indian Institute of Horticulture Research (IIHR) were confirmed to be *R. solanacearum*. While only 2 out of 8 strains collected from chili plants in Banglore and Kerela were confirmed to be *R. solanacearum* using boiled bacterial cells as templates (Fig. 2)

M 1 2 3 4 5 6 7 8 9



**Fig. 2** PCR-based diagnostics of bacterial wilt in India. Lane M: Lamda DNA/EcoR1 & Hind III Marker; Lane 1 to 8: RSCU, RSCV1, RSCV2, RSCK1, RSCK2, RSCK3, RSCK4, RSCK5; Lane 9: positive control (Pss71)

**Indonesia:** Chili pepper plants showing wilting symptoms were collected from Central/West Java and West Sumatra. The six isolates were confirmed to be *R. solanacearum* following the AVRDC protocol (Fig. 3). The protocol was also used to identify strains isolated from tomato and banana.



**Fig. 3** PCR-based diagnostics of bacterial wilt in Indonesia. Lane M: 100 bp marker; Lane 1: positive control (Pss 71 DNA); Lane 2 to 7: CB2, PD2, PD3, DL14, BBS2, and BBS4.

**Thailand:** The Thai partner has extended the use of the protocol to confirm the identity of the strains in their collection from many economic crops (more than 200 strains). Moreover, the protocol has been used to certify ginger lots that are free from the pathogen for export to South Africa with a sample preparation method.

### **Chilli veinal mottle virus (ChiVMV)**

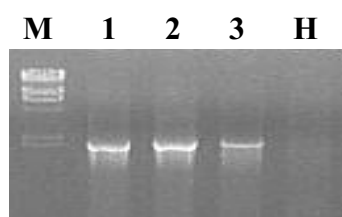
Molecular detection has been developed by reverse transcription PCR (RT-PCR). All 24 isolates (Taiwan 8, India 4, Indonesia 2, China 2, and Thailand 8) were detected using the primer pair Poty3 (5'-TGAGGATCCTGGTG(C/T)AT(A/C)GA(A/G)AA (C/T)GG-3') and Oligo(dT). For the specific detection, the primer pair Oligo(dT) and CVMV1037Pol (5'-AGCATGGAGAGAGCGACATTAGTC-3') was used for RT-PCR, except the isolates Cikabayan2 and Pataruman from Indonesian. The primer pair, Pata-F1/R1 (Pata-F1: 5'-AACCTGAGCGTATAGTTTCA-3'; Pata-R1: 5'-TACGCTTCAGCAAGATTGCT-3') was designed to specific amplify of those two Indonesia isolates.

### **Cucumber mosaic virus (CMV)**

A detection primer pair (CMV-CPF/R; CMV-CPF: 5'-(T/A/C)A(A/G/C/T)(T/C)TTT(G/A/T)(A/G)GGTTCAATTCC-3', CMV-CPR: A(A/G)CTGGA(T/C)GGACAACCCGTTT) have been developed for CMV detection by RT-PCR. However, because it was found to be as efficient as PCR for detection of the virus it was decided to use the cheaper ELISA for virus resistance screening in the breeding program. The National Chung Hsing University, Taiwan has kindly provided anti CMV antibody for the ELISA. The antibody efficiency has been tested at AVRDC and the antibody distributed to the NARS.

### **Begomoviruses causing leaf curl of chili**

The general begomovirus primer pair PAL1v1978/PAR1c715 was found to be effective for the detection of begomoviruses causing leaf curl of chili from 13 countries in Asia, and was recommended for use by the NARS virologists. In India, PCR based diagnosis was developed for chili leaf curl begomoviruses. Two sets of primers specific to the coat protein and replicase genes were designed and tested for chilli-infecting *Pepper leaf curl Bangladesh virus* (PepLCBV) and *Tomato leaf curl Sri Lanka virus* (ToLCSV). The coat protein specific primers amplified about 0.6kb DNA fragment from PepLCBV and ChiLCV-Multan and 0.8kb fragments from ToLCSV. The Replicase gene specific primers amplified 1.0kb fragment from PepLCBV and ChiLCV-Multan and 0.8kb from ToLCSV. These primers could easily differentiate the chili leaf curl begomoviruses in India.



**Fig. 4** PCR based diagnosis of begomovirus causing Chili leaf curl disease using specific primers in India. Line M:  $\lambda$  DNA Hind III + EcoRI digest marker; line 1: isolate from Tamil Nadu; line 2: isolate from Rajasthan; line 3: isolate from Haryana; line H: healthy control.

### 2.1.b Lateral flow diagnostics for viruses (YR1)

A lateral flow system for rapid diagnosis of CMV and ChiVMV has been developed by the cooperators in China and a commercial agent is now being sought who can make these diagnostic strips available for an affordable price.

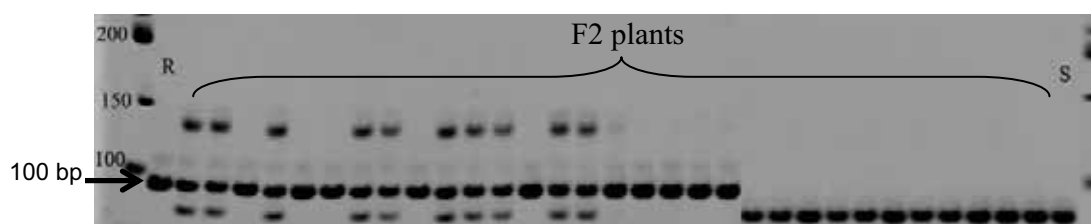
### Activity 2.2 Testing of sequential/simultaneous inoculations with targeted pathogens

An inoculation procedure for screening for resistance to Phytophthora was developed. Five-week-old pepper seedlings were inoculated with *P. capsici* by soil-drenching. Inoculation with anthracnose spores was done at the green mature fruit stage (one week before mature). For screening of pepper lines simultaneously for CMV and ChiVMV sequential screening was standardized. It was found that sequential inoculation of ChiVMV followed by CMV was the most reliable for screening pepper lines for resistance to these two viruses.

## OUTPUT 3: Molecular markers for targeted diseases developed or refined for use

### Activity 3.1 Use of molecular markers for the selection of anthracnose-resistant lines

AFLP marker A518 associated with anthracnose resistance was sequenced. An insertion/deletion (INDEL) was identified differentiating resistant and susceptible genotypes. A primer pair (F: GGTATCTTATTTTCATAGGGACCAGGCA; R: TTTGCGGTAGTGACAACAACCTTTACAGCCA) was designed for a gel-based marker assay utilizing the 10-bp INDEL (Figure 5). Based on the results, this INDEL marker is highly correlated with the original AFLP marker A518 and anthracnose resistance at the green fruit stage. Thus, this marker could be used in marker-assisted selection of pepper genotypes resistant to anthracnose derived from PBC932.



**Figure 5** Acrylamide gel image showing the 100-bp molecular marker associated with pepper anthracnose resistance. R: resistance parent; S: susceptible parent.

### Activity 3.2 Adaptation of available public domain molecular markers for the development of elite pepper varieties with multiple disease resistance

A molecular marker related to the ChiVMV resistance has been identified through QTLs by Dr. J.M Prince, California State University, USA. Two resistance gene loci have been identified and one was located in chromosome 6.

## OUTPUT 4: Mode of inheritance to each of the pathogens determined by classical methods complemented by molecular markers and disease diagnostics

### Activity 4.1 Inheritance of resistance to ChiVMV and CMV, anthracnose, bacterial wilt and phytophthora determined

Crosses between resistant and susceptible parents were made at AVRDC, and seed sets were distributed to pathologists in the several countries. The sets included resistant and susceptible parents, F1, F2, BC1rF1 and BC1sF1. The genotypes used in the cross combinations were as table 1.

**Table 1** The genotypes used in the cross combinations

Country Res. Line	CMV VC246	ChiVMV PBC521, VC58a	BW PBC384 (resist)	Anthracnose IR/PBC932 BC3F6	Phytophthora PI201234
India	Pusa Jwala	Pusa Jwala	Pusa Jwala (M.res)	G-4	Pusa Jwala
Thailand	Phichit1	Phichit1	Phichit1 (susc)	Phichit1	Phichit1
Indonesia	Jatilaba	Jatilaba	Susan's Joy' (susc)	Jatilaba	Jatilaba
China	(no res source)	Pan Family	ZDC (susc)	Pan Family	Pan Family

Evaluations of the populations were attempted both at AVRDC, using the standard pathogen strain, as well as in each cooperating country, using pathogen isolates collected and characterized locally.

### Phytophthora

AVRDC: Inheritance of resistance to phytophthora blight carried by the resistant variety PI201234 was evaluated in crosses with susceptible PLV parents from each of the four target (Pusa Jwala, Pichit-1, Jatilaba, and Pan Family), utilizing the standard isolate representing the severe race 3. (*Pc* 33E-6). Poor results were obtained in 2006, suggesting imperfect test environment, and the test was repeated in 2008, with more credible outcomes. Interpretation, however, continued to be difficult. F<sub>2</sub> generation segregation ratios continue to provide poor fits to any simple inheritance model. F<sub>1</sub> and resistant backcrosses all generally reflect strong dominant expression of resistance, but F<sub>2</sub> and susceptible backcrosses fit single dominant (3R:1S) or recessive epistasis (9R:7S in the F<sub>2</sub>) segregation ratios. The cross between PI 201234 and Phichit comes closest to fitting a single dominant genetic model. Jatilaba X PI 201234 also approximates this pattern, but displays insufficient resistant plants in the F<sub>2</sub>, and too many in the susceptible backcross. The remaining crosses provide a poor fit to a recessive epistatic pattern, but better than other candidate ratios. As has been shown in the array of F<sub>1</sub> hybrid combinations, both resistant and susceptible parents may harbor minor genetic factors that exert significant impact on the expression of resistance.

Indonesia: A local resistant parent 'Cilibang-2' crossed with the local susceptible parent PSPTC-1 produced a relatively poor fit to a 2-gene model of duplicate recessive epistasis.

India: A matrix of four crosses using resistant parents CM334, PI188478, PI201234, and PI201238 crossed with the susceptible parent Pusa Jwala, generated three evaluations consistent with control of resistance by a single dominant gene, and one cross (PI201238 x Pusa Jwala) indicating control by two recessive genes.

### Anthracnose

AVRDC: Resistance introgressed from PBC932 into *C. annuum* (3 backcrosses) was shown to generally fit a 2-gene model of complementary dominant genes (9R:7S) at the green fruit stage, and a different 2-gene model of duplicate recessive genes (7R:9S) at the red fruit stage. Poor correlations suggest that resistance at different maturities is inherited independently.

Indonesia: The population set is developed from the crossing of resistant parent (C-15) and susceptible parents C-2 and C-19. Twenty well-matured-green chili fruits from each plant were inoculated with *C. acutatum* isolates PYK 04 and BGR 027. The result showed that resistance to anthracnose is controlled by multiple genes without maternal effect. The resistance controlling genes are recessive genes. The dominance level is categorized as partial-recessive. The action of resistance controlling genes to anthracnose was additive and dominant, where additive variance is bigger than dominant variance. Broad sense heritability value was high, whereas narrow sense heritability value was moderate. The selection for hybridization of superior chili- *C. acutatum* resistance is better to be performed in further generation.

## Bacterial Wilt

A standard protocol (Annex 1) was developed at AVRDC and used by partners for disease evaluation.

*Indonesia*: Indonesian analysis of resistance to bacterial wilt involved two crosses between resistant parents PBC473 and TitSuper crosses with the susceptible parent PBC67 (MC5). Both parents were provided by AVRDC Bacteriology Unit as resistance source, at 98% and 93% resistant, respectively. Both cross combinations displayed high levels of dominance of resistance in the F1 generation, and high frequencies of resistant plants in the F2 generations. A complex 3-gene model of epistatic interaction was proposed, and was supported by test backcrosses to the susceptible parent. Inconsistent results were ascribed to the conclusion that the susceptible parent PBC67, was not homozygous; because of the anomalous behavior of that parent line, this judgment seems valid. The very high frequency of resistant individuals in virtually all populations of both crosses may result from the unusual susceptibility of the parent PBC67 to the local pathogen isolate, which may have been elicited by a relatively minor offtype which would normally be resistant to other pathogen isolates.

*India*: Resistance to Bacterial Wilt was assayed in the cross between the Indian PLV Pusa Jwala and the resistant parent PBC384. Parental, cross and backcross populations were provided by AVRDC, and screened at IHR using the local *Ralstonia solanacearum* isolate "Kerala RS". Resistance is not fully dominant, and selected segregates should be reevaluated, and reselected once or twice to stabilize the resistance.

## ChiVMV

Duplicate assays at AVRDC of resistance derived from VC58a in each of four PLV backgrounds found good fit ( $p > .05$ ) to a single dominant gene model in six of the eight tests. Broad sense heritability in these six tests varied from .33 to .98, and narrow sense heritability was highly variable, and not informative. Evaluation in Indonesia indicated control by a single dominant gene, with medium to high heritabilities.

## CMV

*AVRDC*: Duplicate assays of resistance derived from VC246 in each of four PLV backgrounds conducted at AVRDC were uninterpretable, because of poor repeatability in the resistant parent, and incongruous segregation ratios in F2 and backcross generations. Fresh seed has been generated, and the tests will be conducted again. In trials in Indonesia, resistance to CMV was found to be controlled by a single recessive gene, but hybrids expressed overdominance for resistance. In India, resistance to CMV reflected control by a single recessive gene. Numerous plants of the resistant parent developed disease symptoms, so the experiment is scheduled for repetition.

## Activity 4.2 Anthracnose markers compared with fruit assays

Two AFLP markers (257 bp [A257] and 518 bp [A518] bands) demonstrated strong association with anthracnose resistance during the green fruit stage (Table 2). The estimated distances from the putative locus for anthracnose resistance are 1.6 and 4.9 cM for A518 and A257, respectively, indicating strong potential for use in marker-assisted breeding application. SCAR (sequence characterized amplified regions) markers for easy routine and high throughput applications are also being developed by AVRDC.

**Table 2** Association of AFLP markers to anthracnose resistance based on average lesion diameter on the fruit

Marker	Group	Mean	N	R2	P value
A518	S	9.43	74	0.72	<0.0001
	R	3.55	170		
A257	S	8.84	76	0.55	<0.0001
	R	3.75	168		

## OUTPUT 5: Selection of multi-disease resistant chili progenies completed and advanced

### Activity 5.1 Combination of multiple disease resistance genes using conventional breeding, molecular markers and improved diagnostics for selection

#### Activity 5.1.a Resistance to 4 diseases confirmed in selections from YR3 trial

**AVRDC:** Advanced lines carrying multiple disease resistance in Project national backgrounds (PLVs), are being evaluated for final selection and increase at AVRDC. These lines have all been screened and reselected under managed disease screens in Taiwan, utilizing aggressive strains of the several pathogens. These lines are being made available to the appropriate national partners, to be evaluated under their conditions. Three lines incorporating resistance to several of the project target pathogens have been included in the 2008 International Chili Pepper Nursery (ICPN18), which is distributed world-wide, to cooperating researchers who will evaluate the lines under their local production environments. As of Mid September, 2008, 42 sets have been sent out from AVRDC. Ten lines, incorporating resistance to three or four of the target pathogens, are currently being tested in AVRDC's 2008 Hot Pepper Preliminary Yield Trial (08HPPYT), and several of these will be chosen for inclusion in the 2009 ICPN19 trial set. Specifications and characteristics of the lines are presented in Annex 2: Multi-disease resistant progenies selected.

**China:** The disease resistance screening of 5 selected advanced lines have been conducted in the Hainan Island (Table 3)

**Table 3** Disease resistance in Chinese candidate lines:

Line	Pedigree	Percent Resistant Plants				
		CMV	CVMV	Bacterial wilt	Anthracnose	Phytophthora
C001	Pan-family large chilli selection X Huangxin early maturing chilli	46	40	76	10	36
C002	West River Early chilli selection X Huangxin early maturing chilli	44	36	80	16	40
C003	Pan-family large chilli Selection X Fu Dijian chilli	38	40	40	14	44
C004	Xiangxi early chilli selection X Zhu Dachang chilli	42	42	44	30	38
C005	8911 chilli selection X Jijiao early chilli Selection	50	44	60	12	32

**India:** Eighty advanced selections (48 at F<sub>7</sub>, others at F<sub>4</sub> to F<sub>6</sub> generations) from CCA crosses were evaluated in the field. Twelve lines displayed yields of at least 100 g/plant. Twelve individual plant selections were made for advance and broader evaluation. 30 additional lines selected from crosses made in India, with non-project sources of resistance, and generated six individual plant selections. Retained resistance to Bacterial Wilt and CMV was evaluated in 20 selections. Wilt resistance ranged from 63 to 100% (average 88.7 +/- 8.7%); CMV resistance ranged from 36 to 100% (average 84.3 +/- 15.2%). One selection displayed 100% resistance to both BW and CMV, and two others were completely resistant to CMV and 90% or more resistant to BW.

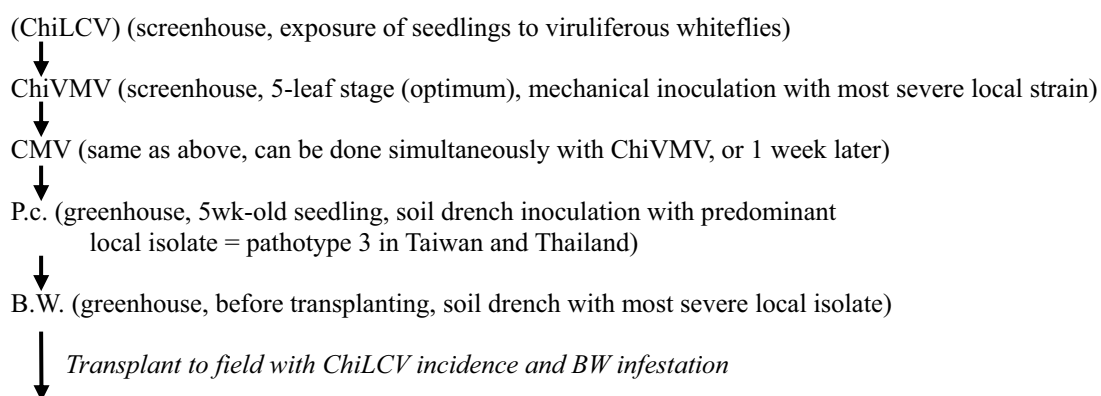
**Thailand:** Despite setbacks in early phases of the project, Dr. Siri Kumphai has actively advanced early generation selections from AVRDC, and has identified two lines which are currently being multiplied, and staged for demonstration to farmers. These lines are F<sub>7</sub> selections from the cross CCA5684, made at AVRDC in 2003, with the pedigree: SriSaket-1/0209-4//SriSaket-1/PBC495. At least partial resistance to two of the four of the project target diseases has been documented (see Advanced line summary Tables, Appendix 1), and it is anticipated that further reselection will be conducted in cooperation with interested farmers in various production regions. Replicated (four reps) performance trials were conducted at three locations, comparing three selected lines to two local check varieties, utilizing production methods similar to those recommended by AVRDC. Dr. Kumphai will be retiring in a few years, but he has involved two other breeders at the Phichit station in managing the project materials, and he expects that they will continue the program in future

variety development, and disease resistance screening. They continue to grow out, screen, and select progenies provided to them from AVRDC, and are crossing promising selections with locally preferred varieties, to improve fruit type.

*Indonesia*: At least four advanced lines have been selected in Indonesia for evaluation at the farmer level, and potential release. Cross combinations were made in Indonesia, utilizing disease resistant lines from AVRDC (ICPN12#4, PBC495, ICPN7#3, CCA321, and 0209-4) and selected parents from the Indonesian breeding program (IPB C2, and IPB C). Farmer evaluations have not yet been completed.

### **Activity 5.1.b Effective simultaneous or sequential inoculation determined in 2.2.a with all pathogens implemented**

The general sequential screening scheme below was proposed at the May 2006 Planning meeting in Changsha, China for segregating populations:



- a) exposure to ChiLCV (whitefly-infected field with high disease pressure)
- b) Anthracnose (incubation room, artificial inoculation of detached green fruit)

<u>Resistant checks</u>		<u>Susceptible checks</u>
CMV:	VC 16	VC 27
ChiVMV:	VC16	VC 27
RS:	PBC 066 (MC4)	PBC 1367
PC:	PI 201234	Early CalWonder
Anthracnose:		
Cc:	PBC 81, 932	Susan's Joy
Cg:	PBC 81, 932	Susan's Joy
Ca:	PBC 81, 932	Susan's Joy

### **Activity 5.2 Improved lines selected by NARS tested in farmers' field under improved management practices and selections advanced through farmer's participation**

#### **Activity 5.2a Seedling vigor and stand establishment improved by starter fertilizer incorporated into production**

*AVRDC*: A balanced fertilization program was developed for chili pepper based on starter solution technology (SST). Results indicated that application of starter solution gave significant booster effects on early plant growth compared with plants fertilized with nutrient-balanced organic fertilizer (NBOF) alone or the standard organic check (SI), e.g. 95-108% and 21% increments in top dry weight for NBOF and SI treatments, respectively. Organic starter solution resulted in 22% more plant weight while organic starter solution

applied with rice hull charcoal gave a 123% increment in plant weight as compared with application of NBOF alone.

Marketable yield of chili (*Jin's Joy selex*) exceeded 36 t/ha as a result of starter solution application. Among organically fertilized plots, those with starter solutions applied at early stages and solid fertilizers side dressed at critical times of plant growth (12 and 38 DAT) yielded 18-24% higher than SI check in the first four harvests. The highest yield was achieved when NBOF was combined with organic starter at transplanting, two additional organic liquid solution 12 and 25 DAT and then side dressed with solid inorganic fertilizers at 38 DAT. Marketable yield was 20% higher than the application of organic fertilizer alone.

Cumulative yields of chili applied with organic starter solution were lower than the SI for the five harvests but exceeded the SI or NBOF plots after six harvests. Cumulative yields of chili applied with NBOF alone were slightly higher than the SI for the first four harvests and decreased since the fifth harvest.

A demonstration trial conducted also showed that application of one starter solution increased marketable yield by 10% compared to use organic fertilizer alone. Application of starter solution 2 to 3 times further increased yield by 18-19%. Two applications of starter solution also enhanced marketable yields by 19% in the plot where chemical inorganic fertilizers were used.

China: Various field studies were conducted on seedling production, influence of sowing dates, effects of micro-fertilizer, NPK fertilizer, and bio-pesticides on yield of chili. Results showed that different sowing periods significantly affected growth and development of chili. The traditional sowing period in early November resulted in better growth and early development (15 days earlier) compared to sowing periods in early January to late February. Number of fruits and total yield of chili sown in early November were 16.7% higher than sowing in January to February. Three applications of micro-fertilizer every 7 to 10 days on chili seedlings improved seedling growth and vigor in terms of root development, leaf and stem growth, high chlorophyll content and increased disease resistance in the nursery. This was also confirmed in the field at transplanting as shown by increased rate of leaf photosynthesis and improved resistance to field diseases (TMV and CMV). Yield increased by 10-18%. Frequency of chemical pesticide and fertilizer application was reduced by 30% and 20%, respectively as a result of micro-fertilizer application.

India: Results indicated cocopeat as growing medium along with three drenching of starter solution at nursery stage was a good practice. Treatment that received starter solution at seedling stage had significantly higher dry chili yield than regular practice of basal application and top dressing 28 days after transplanting. Yield of chili applied with 6 times side dressing was lower by 8.2% compared to treatments applied 6 times with starter solution.

Indonesia: Results indicated that application of inorganic as well as organic starter solution increased chili pepper growth and yield in low fertility soils. The highest marketable yield was obtained with application of standard inorganic fertilizer (SI) + cow manure (CM) + inorganic starter solution (SSGC). With cow manure or standard inorganic fertilizer, addition of organic starter solution reduces total unmarketable yield, improved fruit quality and increased marketable yield.

Thailand: The effect of plug size, growing media and non-woven covering materials with various colors on germination, growth and seedling vigor of chili was investigated. In general, seedling growth and vigor were significantly greater in seedlings grown in 60-cell and 104-cell trays. Seedlings grown in commercial mix growing medium were taller and more vigorous than those grown in peatmoss, coconut coir + compost or coconut coir + sand indicating that the commercial mix growing medium is superior to the other growing media. The effect of starter fertilizer solution on chili growth and yield was evaluated and compared with organic and inorganic fertilizers. Although differences in marketable yield were not significant, treatments applied with starter solution produced yields that are 30 to 50% higher than the control plot applied with cow manure alone.

### **Activity 5.2b      Affordable, simple and water use efficient drip irrigation system evaluated in NARS**

*AVRDC:* The studies on low-cost (affordable) drip irrigation system were conducted to determine the benefits of drip irrigation in terms of water use efficiency and marketable yield as well as fruit quality. A modified low-cost affordable drip irrigation system developed by the International Development Enterprises (IDE) was evaluated for its efficiency and adaptability in chili pepper production. The study also determined the minimum water requirement of chili for optimum production. Results indicated that crop irrigated at -30 kPa produced the highest marketable yield (16 t/ha) and was significantly different from plots under irrigation regimes of -20 kPa (14 t/ha), -50 kPa (12 t/ha) and -70 kPa (11 t/ha). Although optimum yield was obtained at irrigation regime of -30 kPa, water use efficiency was almost similar with -70 kPa. This suggests that chili pepper can be grown at reduced irrigation water using low-cost affordable drip system with no drastic yield reduction. In the following year two chili varieties: CCA 321 and Delicacy 193 were grown under furrow and drip irrigation systems at irrigation regimes (rates) of -20 kPa, -50 kPa and -70 kPa based on soil moisture tension. Results indicated that water use was reduced by 20-90% in drip compared to furrow irrigation. Although yield from furrow-irrigated plots were higher than drip irrigated plots, differences were small and not significant. Incidence of Southern blight disease in chili pepper was higher in furrow irrigated plots compared to drip irrigated plots. Using low-cost drip irrigation system increased water use efficiency 2-8 times compared to furrow irrigation.

*China:* The effect of drip irrigation and flood (furrow) irrigation was compared on growth and yield of chili variety "Xiangyan-10." Drip irrigation was applied immediately after transplanting and continued regularly (at intervals) until 80 days. Furrow irrigation was applied 5 to 7 times during the growing period. Results indicated differences in vegetable growth, development as well as yield. Highest marketable yield was obtained from drip irrigation ranging from 15.0 to 17.5 t/ha, whereas yield from furrow irrigation was in the range of 10 to 13 t/ha. It was also observed that disease incidence (bacterial wilt and fungus) was higher in furrow than drip irrigated plots.

*India:* the low-cost drip irrigation system was compared with furrow irrigation, regular micro-tubing and drip line on their influence on four chili varieties. The five irrigation systems were: 1) furrow; 2) drip-micro-tube; 3) drip regular line with polyethylene mulch; 4) low-cost drip tape; and 5) regular drip line. The results indicated overall superiority of all drip systems compared to furrow irrigation in terms of chili growth and yield parameters. Among the cultivars tested, GDH-57 recorded significantly higher yield (2.15 t/ha dry chili) compared to Pusa Jwala (1.82 t/ha). No significant yield differences were observed among the microirrigation systems evaluated, however, drip irrigation system recorded 18 percent increase in yield compared to furrow irrigation. Polyethylene mulch further enhanced growth and yield parameters of chili varieties.

### **Activity 5.3d      Twenty (20) advanced selections made in each PLV background**

In India, 12 lines were selected displaying resistance to CMV and bacterial wilt, with yields greater than 100g/plant.

Two to three lines have been nominated by each partner country for advanced trials at research stations, and in farmer trials. Repeated screening and selection at AVRDC Headquarters has generated 15 advanced lines displaying resistance to all four target diseases, and 32 with resistance to three of the four diseases. Fifteen lines have been chosen for replicated yield trials, and seed samples have been forwarded to national partners in Thailand and Indonesia for testing in their local conditions.

### **Activity 5.2.e      Three (3) multi-resistant lines in each PLV background tested in uniform trials with GAPs**

*India:* Three multi-resistant lines ( $F_6$  generations) + 2 PLVs were tested in on-station uniform trial using farmers' practice and improved management practices (GAPs). Three

multi-resistant lines with PLV background (GDH-57, CCA 5478 and CCA 5479) were compared with two PLVs (G-4 and Pusa Jwala). Improved management practices adopted were: seedlings raised using bio-pesticide inoculated medium, starter solution, and polyethylene mulch with drip irrigation. Under farmers' practice, 60 cm spaced ridges and furrows were made and irrigation was provided by furrow method. Results of trials indicated that among the cultivars, significantly higher dry chili yield of 2.92 t/ha was recorded in CCA 5478 compared to other cultivars. Between the production system (crop management), drip irrigation + mulching recorded a significantly higher dry chili yield (3.1 t/ha) compared to drip irrigation alone (2.25 t/ha) and farmers' practice (1.82 t/ha). These promising results indicate that multi-disease resistant lines developed from this project along with good crop management practices (GAPs) has the potential of increasing yield of chili pepper by 30 and 60%, respectively.

Indonesia: Five chili improved genotypes with multi-disease resistance developed from several crossings: a) (9x4) x 15; b) (9x4) x 18; c) (9x4) x (2x8); d) (10x8) x(9x4); and e) (2x3) x (9x4) were evaluated by four local farmer cooperators in on-farm trials. The improved genotypes were planted under three crop management practices: 1) plastic mulch with drip irrigation; 2) plastic mulch (no drip irrigation); and 3) farmer's practice (bare soil – no mulch and hand watering – no drip irrigation). In addition to Treatment 1 (plastic mulch + drip irrigation) the plots received starter solution and preventive pest management. This treatment represents good agricultural practice (GAP) package. Based on farmers' observation and evaluation, cultural practices including drip irrigation are too expensive and drip irrigation is not practical during the rainy season when water for irrigation is not a problem. The improved chili genotypes are not preferred by farmers because of larger fruit size (*Cabai Besar*). Farmers prefer the "Keriting" type (*Cabai Keriting*) or "bird's eye" type (*Cabai Rawit*). According to farmers, these local varieties and chili types demand higher market price and have good post-harvest handling features.

Thailand: On-station variety evaluation trials were conducted in three locations: 1) North – Pichit Province; 2) Northeast – Srisaket Province; and 3) West – Kanchanaburi Province. The evaluation trials used four multi-disease resistant lines from AVRDC: 1) CA 5684-758; 2) CCA 5684-5-2-1; 3) CCA 5684-18-3-4; and 4) CCA 5684-31-1. A locally adapted variety "Srisaket 13" (Hua-ruae) from the Department of Agriculture was included in the trials as a local check entry. At the Pichit Horticultural Research Center, some 80 chili growers from Pichit Province evaluated the horticultural traits of the varieties grown in Chili Variety Demonstration Field. Horticultural traits included: plant height, branching, stem vigor, disease tolerance, insect tolerance, fruit set, fruit shape, fruit appearance, pungency, aroma, fruit color (green or red), drying quality and general characteristics. All trials were conducted under good management practices.

Highlights of results indicated that:

- For variety (line) CA 5684-758, most growers preferred the red color of this variety (82%), good drying quality (72%), while preference for disease and insect tolerance received low preference (42.6 and 43.1%, respectively).
- For CCA 5684-5-2-1, most growers preferred the red color of this line (75%), good fruit set (68%), good drying quality (64%), but least preferred in terms of plant height (13%).
- For CCA 5684-18-3-4, growers preferred the branching trait of this variety (66%), plant height (59%), and stem vigor (59%). The least preferred trait of this variety is the aroma (28%).
- For line CCA 5684-31-1, plant characters preferred by most growers are good fruit set (53%) and the red fruit color (50%). Plant height is the least preferred trait for this variety (22%).
- The locally adapted variety Srisaket 13 (Hua-ruae) was the most preferred variety by farmers. Growers preferred almost all plant and fruit characteristics of this variety. The least preferred trait of this variety is the aroma (35%). In terms of disease and insect tolerance this variety was comparable with lines CA 5684-758 and CCA 5684-18-3-4.

### **Activity 5.2.f      Advanced lines evaluated using locally adapted GAPs**

*India:* Three multi-resistant lines were grown under good agricultural practices (GAPs) consisting of protray seedling production, bio-agent seeding inoculation, application of starter solution, mulching and drip irrigation. This plot was compared with farmer's practice established on adjacent field. (The results have not been received from India at the writing of this report).

*Thailand:* 80 chili growers evaluated 13 plant traits (characteristics) of improved lines of chili and a locally adapted variety. The growers scored the traits for all varieties according to their preference. The plant characters evaluated were plant height, branching, stem vigor, disease and insect tolerance, fruit set, fruit shape, fruit appearance, pungency, aroma, fruit color, drying quality and general characteristics. Results of the evaluated indicated that the locally adapted variety "Srisaket" was the most preferred variety and the improved line CCA 5684-31-1 was the least preferred variety. These lines were evaluated under good agricultural practices (GAPs).

### **Activity 5.3      Phytophthora and begomovirus resistant sources crossed with promising progenies carrying multiple resistance**

#### **ChiLCV**

At AVRDC, 62 F4 progenies have been selected from crosses of PLVs with the ChiLCV resistant parent 'Lorai', and 90 F5 progenies from crosses between PLVs and several candidate sources of begomovirus resistance. Seed has been forwarded to cooperators in India, Thailand, and Indonesia for screening under infective conditions.

#### **Phytophthora**

Hybrid F1s and segregating populations for resistance to Phytophthora blight generated from 4 cross combinations which were 4 resistant parents (PI201234, CM334, PI201238 and PI188478) crossed with 4 PLV (Pusa Jwala, Pichit-1, Jatilaba and Pan Family) from each targeted country. The evaluation for disease resistance to most predominant pathotype 3 (*Pc* 33E-6) was conducted.

## **9.      Assessment of Research findings**

Chili pepper (*Capsicum annuum*) is a major crop for small and resource-poor farmers in Asia. Due to prevalence of viral, bacterial and fungal diseases, and lack of cultivars with multiple disease resistance, the yields are extremely low (about 5.5 t/ha compared to 10–17 t/ha elsewhere) in these countries. Six major diseases contributing to low yields have been addressed in the project including the *Cucumber mosaic virus* (CMV), *Chilli veinal mottle virus* (ChiVMV), bacterial wilt (BW; *Ralstonia solanacearum*), anthracnose (*Colletotrichum* spp.), Phytophthora blight (*Phytophthora capsici*) and begomoviruses causing chili leaf curl disease. PCR-based methodologies have been developed for the pathogen diagnostics. Especially, the specific detection primers for all pathogen and the lateral flow diagnostics for viruses could be used in rapid identification and assistance of the breeding program. The standardized-procedure of resistance screening used in different countries also contributes to the reliable results of cultivars developed with multi-disease resistance. To assist the breeding process, pathogen-resistance-related-makers have been developed for anthracnose and ChiVMV. These could be used to accelerate the breeding program in the future. Also, resistant lines have been identified for other pathogens (including begomoviruses, phytophthora and bacterial wilt) and these could be used for maker development in the future.

Conventional pathology and breeding methods were applied to good effect. Appropriate resistant germplasm was identified, methods for coordinated screening and selecting for multiple traits through segregating generations were established, and advanced lines were generated in most countries that combine some or all of the target characteristics. The yield and fruit type of submitted selections may not exceed those of the background standard

varieties. However, methods have been provided which should allow researchers in national programs to advance their breeding programs, with additional cycles to locally produced varieties that are well adapted to the local conditions and challenges. Preliminary combinations of additional disease resistances (e.g. against phytophthora) to the multi-resistant selections moves the program forward toward continually more robust varieties.

In addition, the integration of improved crop management practices (starter solution, growing media, bio-fertilizers, bio-pesticides and drip irrigation) with chili production will impact chili production in the major chili producing countries. In all trials conducted, both local and improved chili varieties produced higher yield when good agricultural practices (GAP) were used. The adoption of good agricultural practices will greatly impact chili production in the countries.

In this multi-disease resistant chili project (Phases I and II), a team-work approach has been established within and between cooperating institutions in China, India, Indonesia, Thailand and with AVRDC to integrate the several disciplines and resources to improve farmers' lives through improved chili production. Sources of disease resistance have been shared internationally; similarities as well as distinctions between pathogen types and aggressiveness in associated countries have been characterized. By the team-work, the country information and technologies have been exchanged and updated. These accomplishments should help to extend our understandings and technologies more broadly to other countries and regions. Collaborative teamwork in this project was a novel strategy for some of the member NARS and the improvement in their effectiveness can be used as an example for other crops, applications, and goals.

## 10. Know-How Transfer

1. Research methodology on isolation and identification of *R. solanacearum*, the bacterial wilt pathogen, as well as disease screening has been transferred and adopted by project partners.
2. The resistance sources of anthracnose, PBC932 and 81 were disseminated to both public and private sectors for use as resistant parents in breeding programs.
3. The technique by using microinjector to evaluate the anthracnose resistance is suitably reliable and has been transferred to NARS to conduct the resistance screening.
4. Three species specific-primers (CaINT2/ITS4, CgINT/ITS4, and CcINT/ITS4) and ITS-RFLP fingerprinting technology have been transferred to all NARS for the identification of *C. acutatum*.
5. The resistance sources of *Phytophthora capsici*, CM331, CM334, PI189550, PI201232, PI201234, and PI20138 have been disseminated to all NARS.
6. The identification techniques of *Phytophthora capsici* isolates by species-specific primer have been transferred to the NARS.
7. PCR primers for detection begomoviruses causing Chili leaf curl disease have been distributed to NARS.
8. The technology of molecular identification of ChiVMV have been distributed to NARS and published in an international journal.
9. Improved crop management technologies developed at AVRDC including starter solution, simple and affordable drip irrigation system and containerized transplant production have been transferred to NARS.
10. Through training workshops, action research and field experimentation, knowledge and techniques were demonstrated and successfully implemented in both researcher and farmer-participatory on-station and on-farm trials.

## 11. Training

Date	Location	Objective	No. of persons trained
19-20 May, 2005	Plant Protection Institute, Hunan, China	PCR identification of <i>R. solanacearum</i> using primer pair AU759/760	4 NARS
20 May, 2005	Hunan Pl. Prot. Inst., China	Phenotype and molecular characterization of <i>Colletotrichum</i> spp.	7 NARS of China, India, Indonesia and Thailand
1-31Jan, 2006	AVRDC	Study on chili Phytophthora blight	Mr. Penjore, K. Natural Resources, Training Institute, Bhutan
1-30 Sep,2006	AVRDC	Research techniques of chili anthracnose and Phytophthora blight	Ms. Elvi Sujana Zeinuddin from Nuhems, Indonesia
11-15 Dec, 2006	AVRDC	Study on pepper anthracnose	Ms. Chinnaswamy, N. Univ. of Agri.Sciences, Bangalore, India
03 Jan, 2007	Kamphaeng Saen Kasetsart Univ., Thailand	Advances in the research on chili anthracnose and Phytophthora blight. 25 <sup>th</sup> ARC Training course	14 training scholars from 5 countries
24 Mar -5 Apr, 2007	AVRDC	Characterization of <i>Pc</i> and <i>Colletotrichum</i> isolates	Dr. widjaja, Euis, S. IVEGRI, Indonesia
21 May, 2007	Hunan Pl. Prot. Inst., China	Pathogen characterization and breeding for resistance to pepper anthracnose	20 scientists from Hunan PPI and Veg. Res. Inst.
04-15 Jun, 2007	AVRDC, Taiwan	Training course on screening for resistance to pepper anthracnose and Phytophthora blight	10 pathologists of 8 private sectors of APSA
04-11 Aug, 2007	Pyongyang Veg. Res. Inst., North Korea	Research techniques of chili Phytophthora blight	22 scientists of North Korea
5-18 Aug, 2007	AVRDC, Taiwan	Resistance screening on chili anthracnose	Dr. Ratiya Pongpisutta Kasetsart Univ., Thailand
1 Oct- 31Dec, 2007	AVRDC, Taiwan	Resistance screening for major fungal disease in peppers	Dr. Selvakumar Rajan Central Agri. Univ. India
04 Jan, 2008	26 <sup>th</sup> ARC Training course: Kamphaeng Saen Kasetsart Univ., Thailand	Advances in the research on chili anthracnose and Phytophthora blight	13 training scholars from 6 countries
07Jan, 2008	Maejo Univ. Chiangmei, Thailand	Characterization of <i>P. capsici</i> and host resistance for disease control	20 graduate students and professors from Maejo Univ.
02 Mar, 2008	IVEGRI, Indonesia	Characterization of <i>Colletotrichum</i> spp. and host resistance for pepper anthracnose control	15 scientist from IVEGRI
16-30 Jun, 2008	AVRDC, Taiwan	Resistance screening for resistance to chili anthracnose and Phytophthora blight	Mr. Burai, East-West Seeds in Indonesia
16 Jul, 2008	Veg. Res. Inst. of Hunan AAS, China	Pathogen characterization and breeding for resistance to pepper Phytophthora blight	25 scientists from Veg.Res.Inst. and Hunan PPI
14 Jul, 2006	Bogor, Indonesia	Improved crop management practices for chili production ; Focus group discussion on chili improved cultivation methods: transplant production, fertilizer, drip irrigation and IPM	24
14 Mar, 2008	IHR, Bangalore, India	Farmers' Field Day; Demonstration of good agricultural practices for chili production	50
19-21 Mar, 2008	Pichit Horticultural Research Center, Pichit Province, Thailand	Farmers' Field Day ; Farmer evaluation of improved chili lines in terms of plant characters	80

## 12. Lessons Learned

1. Colonies of *R. solanacearum* could be confused with other bacteria on the semi-selective medium, TTC. Use of the PCR identification method has improved the efficiency of isolation.
2. Use of F2 and backcrosses F1 is not suitable to conduct inheritance study for chili pepper bacterial wilt. AVRDC will follow up using F3 and backcross F2 family lines for the purpose.
3. Although the four countries agreed to implement the activities under crop management, not all of them followed what was outlined in the work plan.

4. Drip irrigation was not evaluated in Thailand since according to country partners this technology is less important among chili farmers.
5. Crop management activities were implemented by four countries except China. In the second phase, there was poor communication links with Chinese collaborators. No report was received on crop management activities. This is what we learned working with our Chinese counterparts. We hope that this can be improved in the future with new projects.
6. We learned that there is some level of interest in the three countries in improving chili production using improved containerized transplant (seedling) production, application of starter solution technology and balanced fertilization program. Positive results were obtained from Indonesia and India showing the benefits of starter solution at seedling stage and at later stages of growth resulting in higher yield compared to plots with no starter solution applied. Even the results in Thailand were not significant, overall yield of chili applied with starter solution was higher than without starter solution.
7. Significant positive result was observed using drip irrigation on chili production in India. All plots under drip irrigation had better yield than plots under furrow irrigation. Integration of mulching with drip irrigation further enhanced growth and yield of chili pepper.
8. Farmers' enthusiasm on improved chili lines/varieties and good agricultural practices was shown by their active participation and attendance in field days and training workshops. However, in Indonesia and Thailand farmers tended to prefer locally adapted varieties due to their superiority in terms of fruit qualities and market price. In India, one improved advanced line GDH-57 was superior to preferred local varieties (PLVs) G4 and Pusa Jwala.
9. The benefits of integrating GAPs were observed regardless of variety. Both locally adapted and improved lines performed better when grown using good agricultural practices compared to farmers' traditional practice.
10. There is a need to evaluate more advanced lines using locally adapted good agricultural practices. The number of entries that were evaluated in India, Indonesia and Thailand was not sufficient to draw final conclusions and recommendations.

### 13. Future Research needs

1. As chili is infected by multiple pathogens and detection of all pathogens simultaneously is required for effective management of diseases. New technologies such as DNA microarrays should be developed for pathogen detection.
2. For the resistance screening of bacterial wilt, using stem necrosis or pathogen colonization as selection criteria under lower temperature environment was suggested by the project. This should be confirmed to provide more flexibility in designing multiple diseases screening scheme.
3. For the breeding, incorporation of phytophthora and leaf curl virus resistance in the advanced breeding lines and development of molecular markers linked to major disease resistance genes are the major issue in the future.
4. The improved varieties developed in this project need to be evaluated under farmer practices and in different agro climatic conditions so as to see the potential and performance of improved lines with respect to multiple disease resistance.
5. Climate change is likely to influence pathogen evolution and diversity and to keep pace with this more disease resistant chili varieties and selections need to be identified. There is thus an urgent need to develop molecular markers linked to the resistance to begomoviruses, tospoviruses, *Cucumber mosaic virus* and chili veinal mottle viruses.
6. In India, the *Groundnut bud necrosis virus* and Capsicum chlorosis viruses (CaCV) are increasing in incidence and contributing to epidemics and yield loss of chili. There is a requirement to develop improved diagnostics and suitable management tactics for these tospoviruses, especially in resistance screening.
7. The improved crop management technologies and GAPs have not been sufficiently applied and evaluated with the improved multi-disease resistant lines developed in this

project. This is an activity that needs to be further implemented. The integration of improved crop management practices with improved multi-disease resistant chili lines would reduce production inputs and ultimately increase yield and productivity.

#### 14. Publications and reports and conference presentations (1-3p.)

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2. Dissataporn, O., Krung S. and Parichat D. 2008. Chili management practices with starter solution. Department of Agriculture and Kasetsart University, Bangkok, Thailand.
3. Dissataporn, Or, Krung S. and Parichat D. 2008. Effect of plug tray size, type of media and shading on seedling vigor of chili pepper. Department of Agriculture and Kasetsart University, Bangkok, Thailand.
4. Gniffke, P. A., Green, S. K., Wang, J. F. and Wang, T. C. 2005. Improving chili peppers for developing countries. Hort Science 40:980.
5. Gniffke, P. A., Lin, S. W. and Wang, T. C. 2007. Evaluation of diverse chili pepper sources for resistance to anthracnose. First International Symposium on Chili Anthracnose held at Seoul National Univ. Korea. 17-19 September 2007, P.42.
6. Gniffke, P. A., Lin, S. W., Wang, T. C. and Liu, C. A. 2007. Development of anthracnose resistant chili pepper varieties at AVRDC – The World Vegetable Center. First International Symposium on Chili Anthracnose held at Seoul National Univ. Korea. 17-19 September 2007, P50.
7. Gniffke, P. A., Wang, J. F. and Wang, T. C. 2005. Improving chili pepper for developing countries. Paper presented in the 102<sup>nd</sup> Annual Conference of ASHS at Las Vegas, Nevada, Jul. 18-21, 2005.
8. Kantharaju, H.M., Krishnareddy, M. And Madhavi Reddy, K. 2008. Identification of *Potyvirus* Infecting Chilli pepper in South India. Indian Journal of Virology (Accepted)
9. Lakshminarayana Reddy, C. N. 2006. Molecular characterization of *Chilli veinal mottle virus* infecting chilli (*Capsicum annum* L.). Ph. D. thesis submitted to Department of Plant Pathology, University of Agricultural Sciences, Bangalore, India.
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## **Annex 1: Protocol for conducting inheritance study on resistance to bacterial wilt in chili peppers**

### **Objectives**

To determine possible mode of genetic control of resistance in chili pepper variety PBC384 to *Ralstonia solanacearum* (Rs) under different genetic backgrounds, strains, and environments

### **Materials and Methods**

- Plant materials

The generations of each cross between PBC384 and Preferred Local Varieties (PLV) are listed in Table 1. The generations of each cross include, two parental lines (P1 as PBC384 and P2 as the other parents), F1 (use P2 as female parent), F2 and backcrosses to both parents (use F1 as female parents).

- Seedling preparation

1. Seed treatment: Soak seeds in 5X Clorox (which is 1.2% of sodium hypochlorite) for 5 min and rinse under running water for 15 min.
2. Direct sow 1 or 2 treated seeds in individual pots (upper diameter: 6.5 cm; bottom diameter: 5 cm; height: 5.5 cm). Seeds are placed in a pit of 1-cm depth and covered with peat moss. Thin to one seedling per pot when the cotyledons are fully expanded and first true leaf emerged. The potting mixture used is prepared by AVRDC farm (use locally available conducive potting mixture).
3. Fertilize seedlings with 500X of foliar fertilizer (BASF Foliar Nitrophoska) once a week after thinning and stop at least 4 days before inoculation.

- Inoculation

1. Strain: Pss71 (used at AVRDC), and other local strains selected by partners. The local strain used should be aggressive and predominant.
2. Inoculum preparation: Streak the Rs strain from storage culture on TTC plates. Select several fluidal colonies with red or pink center and transfer to a 523 plate (or other rich media, such as NA). Incubate the plate at 30°C overnight. Prepare a dense suspension from the overnight 523 culture. Spread 0.1 ml of the bacterial suspension on one 523 plate and keep at 30°C for 24 hours. Transfer bacteria mass from 24 hour-old cultures and suspend in water. Adjust the concentration until the O.D. value reach 0.3 at the wavelength of 600 nm (or about  $10^8$  cfu/ml). If facility of spectrophotometer is not available, the turbidity of bacterial suspension can be estimated by McFarland's scales.
3. Inoculate the seedlings at 3 to 5 true leaf stage (about 4 weeks after sowing). Reduce the soil moisture in pots by skipping one watering before inoculation. Sever one side of the roots by using a knife cutting into soil at 1 - 2 cm away from stem base. Pour 25 ml of the above suspension on the soil surface of each pot right after root severing. There should be no suspension leaking out. The ratio between bacterial suspension and potting mixture is about 1:10 (v/v).
4. Keep the inoculated seedlings at an environment with temperature ranged from 26 to 28°C and relative humidity should be lower than 90%.

- Experiment design

Randomized complete block design with 8 replications. The layout of each replication is shown below.

- Data collection

Severity score (SC) of each plant should be collected every 3 to 4 days until 24 days after inoculation. Thus, there will be 7 ratings for each plant. The severity score ranged from 0 to 5, where 0 means no symptom; 1 means one leaf wilted or defoliated; 2 means 2-3 leaves wilted or defoliated; 3 means more than 3 leaves wilted or defoliated; 4 means all leaves wilted or defoliated; 5 means plant death (dried or collapsed). Record the data in an Excel file and sent to Jaw-Fen Wang (jaw-fen.wang@worldveg.org). Take note of other symptoms, such as leaf necrosis, stem lesion, yellowing.

Table 1. Plant materials used in inheritance studies of PBC384 at AVRDC and partners

Origin of P2	Code	Code/pedigree	Source	Generation
Thailand	PBC384		0530:1-1	P1
	Phichit-1		0530-4-2	P2
	CCA7527	PBC384/Phichit-1	0530:1-1/4-2	F1
	CCA7527 F2	PBC384/Phichit-1	0610-26-1	F2
	CCA7851	CCA7527/Phichit-1	0610:26-1/9-2	BC1s
	CCA7852	CCA7527/PBC384	0610:26-1/23-3	BC1r
India	PBC384		0530:1-1	P1
	PusaJwala		0530:3-2	P2
	CCA7526	PBC384/PusaJwala	0530:1-1/3-2	F1
	CCA7526 F2	PBC384/PusaJwala	0610-25-1	F2
	CCA7849	CCA7526/PusaJwala	0610:25-1/6-2	BC1s
	CCA7850	CCA7526/PBC384	0610:25-1/23-2	BC1r
China	PBC384		0610-23-2	P1
	ZDC (China)		0610-22-1	P2
	CCA7831	PBC384/ZDC (China)	0610:23-2/22-1	F1
	CCA7831 F2	PBC384/ZDC (China)	0710-15-2	F2
	CCA9527	CCA7831/PBC384	0710:15-2/5-2	bc1r
	CCA9526	CCA7831/ZDC (China)	0710:15-1/3-2	bc1s

**Experiment layout (one flat as a replication)**

		Column							
		1	2	3	4	5	6	7	8
Row	5	bc1r-1	F1-1	R.ck-2	F2-7	bc1r-3	Bc1s-4	bc1r-5	S.ck-3
	4	F2-2	P1-2	F2-4	F2-6	F2-9	P1-3	F2-12	Bc1s-6
	3	P1-1	Bc1s-1	S.ck-1	P2-2	Bc1s-3	F2-10	F1-3	P2-3
	2	F2-1	R.ck-1	bc1r-2	F2-5	F1-2	R.ck-3	Bc1s-5	bc1r-6
	1	P2-1	F2-3	Bc1s-2	S.ck-2	F2-8	bc1r-4	F2-11	F2-13

Flat size: 50 cm × 34 cm × 7 cm (L×W×H); If flat is not available, place seedlings of each replication together as a unit and leave sufficient space between units.

		Column							
		1	2	3	4	5	6	7	8
Row	5	6	3	8	4	6	5	6	7
	4	4	1	4	4	4	1	4	5
	3	1	5	7	2	5	4	3	2
	2	4	8	6	4	3	8	5	6
	1	2	4	5	7	4	6	4	4

		Each flat	8*flat
1	P1 sus.	3	24
2	P2 res.	3	24
3	F1	3	24
4	F2	13	104
5	Bc1s	6	48
6	bc1r	6	48
7	S. ck (PBC1367)	3	24
8	R. ck (PBC066)	3	24

## Annex 2. Inheritance of disease resistance study

### Output 4: Evaluation of Inheritance of disease resistances at AVRDC

Resistance to four major diseases of pepper was evaluated at AVRDC and at cooperating national centers over the period of the project's phase II. AVRDC-breeding was charged with generating the various seed lots needed for these studies. The basic approach was to grow out samples of parents, F<sub>1</sub>, F<sub>2</sub> and backcross (both to the resistant and susceptible parents) populations under the standardized screening protocols that have been developed by AVRDC pathologists and collaborators in the various countries. In the cases of CMV and ChiVMV, plants were mechanically inoculated and evaluated two weeks later; In the cases of bacterial wilt and phytophthora blight, root masses of seedlings were drenched with isolate suspensions, and seedlings evaluated for foliar wilt and stem necrosis symptoms, subsequently over one to three weeks; Because of the long growing time needed to produce fruits for evaluation of Anthracnose resistance, inheritance evaluation was studied in separate trials at AVRDC, but not formally at national programs. Aggressive isolates of the pathogens were chosen to induce strong differentiation between resistant and susceptible individual plants. Results presented here are as generated at AVRDC and several of the contributing partner countries; additional results from national cooperators, and overall analysis, will be forthcoming upon completion of their evaluations.

#### Activity 4.1: Inheritance of Bacterial wilt resistance determined against different strains and under different environments at each country

##### 1. Research results

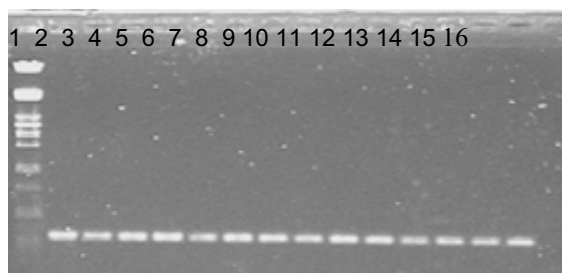
###### Use of specific primers for diagnosing *R. solanacearum*

- *Background*

Isolation of *Ralstonia solanacearum* is normally done using a semi-selective medium named TTC. However, colonies of the pathogen on TTC plates can be confused with other saprophytic bacteria. A species-specific primer pair, AU759/760, has been developed by Opina *et al* (1997). During Phase 1 of this project, difficulties were encountered by collaborators in identification of the pathogen. Thus, it is decided to disseminate this methodology in Phase 2. During the Phase 2 planning workshop, collaborators from China, India, Indonesia and Thailand were trained. And primers and control DNA were provided to each partner.

- *China*

Total of 20 isolates collected from chili pepper plants grown in the disease nursery of Hunan Vegetable Research Institute were confirmed to be *R. solanacearum* following the boiled cell protocol (Fig. 1).



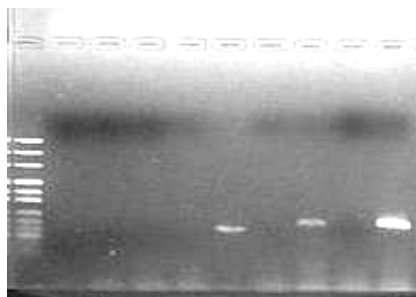
**Figure 1.** Lane 1: Lambda DNA/PstI marker; Lane 2 to 14: Strain Vc58a-8-1-1 (19), VcB58a-8-1-1 (18), CCA5731 (17), P12012-34 (16), CCA5781 (15), PBC137 (14), Vc246 (13), Vc246 (13), PI2012-34 (16), CCA5409 (12), ZCPN12-4 (11), PBC602 (10), CCA5731 (17); Lane 15: positive control (Pss71); Lane 16: negative control

- *India*

Using the AVRDC protocol, 20 strains isolated from wilted tomato plants grown in bacterial wilt disease nursery at Indian Institute of Horticulture Research (IIHR) were confirmed to be *R. solanacearum*. While only 2 out of 8 strains collected from chili plants in Bangalore and

Kerela were confirmed to be *R. solanacearum* using boiled bacterial cells as templates (Fig. 2)

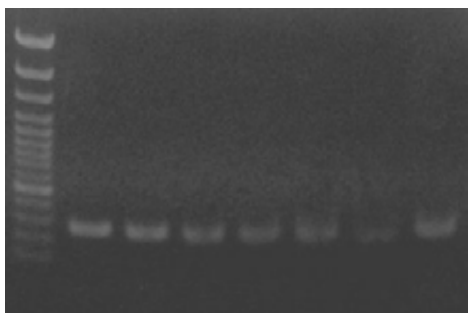
M 1 2 3 4 5 6 7 8 9



**Fig. 2.** Lane M: Lamda DNA/EcoR1 & Hind III Marker; Lane 1 to 8: RSCU, RSCV1, RSCV2, RSCK1, RSCK2, RSCK3, RSCK4, RSCK5; Lane 9: positive control (Pss71)

- **Indonesia**  
Chili pepper plants showing wilting symptoms were collected from Central/West Java and West Sumatra. The six isolates were confirmed to be *R. solanacearum* following the AVRDC protocol (Fig. 3). The protocol was also used to identify strains isolated from tomato and banana.

M 1 2 3 4 5 6 7



**Fig. 3.** Lane M: 100 bp marker; Lane 1: positive control (Pss 71 DNA); Lane 2 to 7: CB2, PD2, PD3, DL14, BBS2, and BBS4

- **Thailand**  
The Thai partner has extended the use of the protocol to confirm the identity of the strains in their collection from many economic crops (more than 200 strains). Moreover, the protocol has been used to certify ginger lots that are free from the pathogen for export to South Africa with a sample preparation method.

#### Inheritance of BW resistance determined against different strains and under different environments at each country

- *Evaluation of resistance to bacterial wilt using different rating systems – a preliminary study for inheritance studies*

#### **Background**

In order to develop the protocol for inheritance studies of bacterial wilt, a set of chili pepper lines with different degree of resistance to bacterial wilt were selected for this experiment. The entry included PBC 384, the stable resistance sources, which is used as the resistant parent in the crosses made for inheritance studies. All entries were grown in 2"pot and inoculated with Pss71 by root severing and drenching method at 4 to 5 true leaf stage. Plants were rated at 3, 7, 10, 14, 21 and 28 days after inoculation (DAI). At 28 days after inoculation, plants showing no visual symptoms were harvested and the colonization of the pathogen at stembase and

mid-stem point were assayed by printing the stem cut on SM-1 plates. The degree of colonization was rated with a 0 to 4 scores.

### **Results and discussions**

The rating system based on severity score and colonization score at mid-stem point were considered suitable for inheritance study. The colonization score at stembase did not provide large difference between resistant and susceptible entries. The system based on days to wilting (reach severity score of 3) can be improved by rating plants every 3 or 4 days.

Table 1. Evaluation of chili pepper for resistance to bacterial wilt with different rating systems

Entry	Severity score <sup>1</sup>	Colonization score (B) <sup>2</sup>	Colonization score (M) <sup>2</sup>	Days to wilting <sup>3</sup>
PBC384	0.0 ± 0.0 <sup>4</sup> c <sup>5</sup>	2.8 ± 0.3 bc	1.1 ± 0.5 c	0.0 ± 0.0 d
PBC066	0.4 ± 0.4 c	2.1 ± 0.3 c	0.9 ± 0.4 c	0.3 ± 0.2 cd
Chinda Type	2.0 ± 0.5 b	3.4 ± 0.3 ab	2.5 ± 0.2 b	1.0 ± 0.4 bc
PBC715	0.7 ± 0.6 c	2.1 ± 0.8 c	1.0 ± 0.8 c	0.6 ± 0.5 bcd
PBC142	2.3 ± 1.2 b	3.4 ± 0.5 ab	2.5 ± 1.3 b	1.3 ± 0.7 b
ZDC	3.7 ± 0.7 a	4.0 ± 0.0 ab	4.0 ± 0.0 a	2.4 ± 0.7 a
PBC1367	4.6 ± 0.3 a	4.0 ± 0.0 a	4.0 ± 0.0 a	3.1 ± 0.5 a

<sup>1</sup> Plants were rated with severity score (0 ~ 5) 28 days after inoculation (DAI). 0 = no symptoms; 1 = 25% leaf wilted; 2 = 50% leaf wilted; 3 = 75% leaf wilted; 4 = 100% leaf wilted; 5 = plant collapsed and died.

<sup>2</sup> Colonization of the pathogen was determined at stembase (B) or at mid-stem point (M) 28 DAI with a printing method. Scores (0 ~ 4) were assigned on each print based on the area covered by bacterium mass, i.e. 0 = no growth; 1= bacteria covered area ≤12.5% of print; 2= 12.5% < bacteria covered area ≤ 25% of the print; 3= 50% < bacteria covered area <100% of the print; 4= bacteria covered the print fully.

<sup>3</sup> Based on the days when plants reached severity score of 3, each plant was assigned a score, i.e. 0 = plants with severity score < 3 at 28 DAI; 1= plants with severity score ≥ 3 at 28 DAI; 2= plants with severity score ≥ 3 at 21 DAI; 3= plants with severity score ≥ 3 at 14 DAI; 4= plants with severity score ≥ 3 at 10 DAI; 5= plants with severity score ≥ 3 at 7 DAI; 6= plants with severity score ≥ 3 at 3 DAI.

<sup>4</sup> Means and standard deviations calculated from 36 plants of each entry.

<sup>5</sup> Means followed with the same letter were not significantly different based on DMRT at 0.05 level.

- *Inheritance studies on bacterial wilt resistance in PBC384*

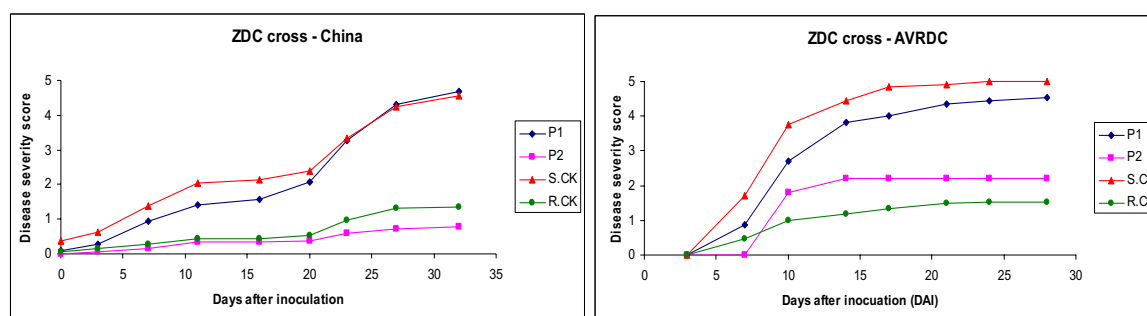
### **Background**

During Phase 1, resistance to bacterial wilt in a chili pepper variety PBC384 was shown to be stable against different *R. solanacearum* strains based on evaluation results conducted at AVRDC and partner countries. Studies to determine possible genetic control of resistance in PBC384 to *R. solanacearum* under different genetic backgrounds, strains, and environments was conducted at AVRDC, China, India, and Thailand. The information could be used by breeders in designing breeding strategies for disease resistance. Crosses were made using PBC384 as the female parent and preferred local varieties nominated by partners as the male parents. Generations of resistant parent (P1), susceptible parent (P2), F1, F2 and backcross F1 to each parent were prepared (see Table 1 in Attachment 1). A standard protocol (Attachment 1) was developed at AVRDC and used by partners for disease evaluation.

## Results and discussions

### Results on ZDC cross

The inoculations were conducted at Hunan Plant Protection Institute, China and AVRDC following the same protocol. In China, the first inoculation failed as no visual symptoms were observed. And a 2<sup>nd</sup> inoculation was conducted when seedlings were 60 day-old. When examining the disease progress curves, it is clear that disease pressure in China trial was much lower. Reactions of the 6 generations tested were very different between the China and AVRDC trials. First, disease severity on F<sub>1</sub> plants was closer to the susceptible parent in the China trial, but closer to the resistant parent in the AVRDC trial. Second, larger proportion of F<sub>2</sub> and backcross progenies had similar reactions as the susceptible parent, while the opposite was observed at AVRDC trial.



Hunan, China				AVRDC, Taiwan			
Strain	PI 2012-34			Strain	Pss 71		
Genotype	Score (32 DAI after 2 <sup>nd</sup> inoculation)*			Genotype	Score (28 DAI)		
P1 (PBC384)	0.78			P1 (PBC384)	2.20		
P2 (ZDC)	4.69			P2 (ZDC)	4.52		
F <sub>1</sub> (CCA7831)	3.04			F <sub>1</sub> (CCA7831)	2.23		
<b>score</b>	<b>F<sub>2</sub></b>	<b>BC1P2</b>	<b>BC1P1</b>	<b>score</b>	<b>F<sub>2</sub></b>	<b>BC1P2</b>	<b>BC1P1</b>
0	0	0	0	0	24	4	15
1	0	0	2	1	20	3	11
2	11	5	13	2	18	10	5
3	54	30	17	3	8	4	1
4	25	7	3	4	3	3	0
5	1	0	0	5	18	11	10
Total No. of plants	91	42	35		91	35	42

\* 2<sup>nd</sup> inoculation was done when seedlings were 60 day-old

### Results on Pusa Jwala cross

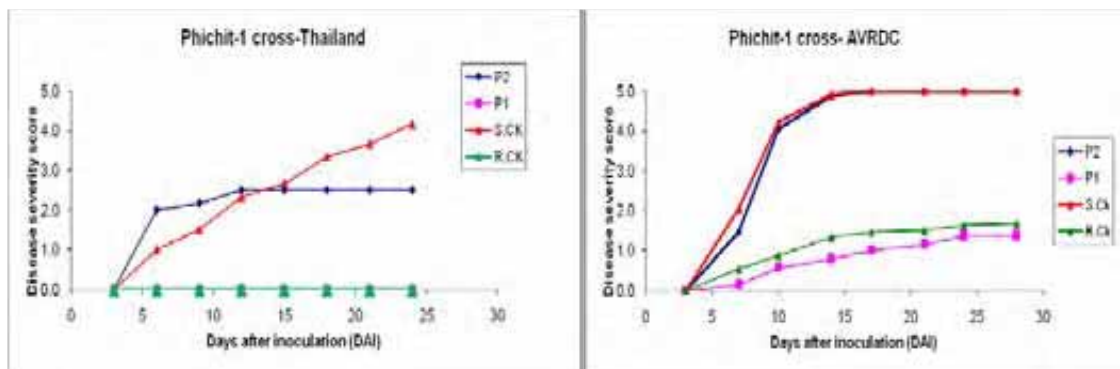
Despite two inoculations, very low proportion of plants showed visual wilting symptom in the IHR trial. Therefore, each plant was scored as “positive” or “negative” based on the presence or absence of the pathogen detected in the collar region.

IIHR, India				AVRDC, Taiwan			
Strain	A strain isolated from chili in Kerala (inoculation conducted twice at 34 and 54 days after sowing)			Strain	Pss 71		
Genotype	Reaction* (~28 DAI)			Genotype	Score (28 DAI)		
P1 (PBC384)	Negative			P1 (PBC384)	0.8		
P2 (Pusa Jwala)	Positive			P2 (Pusa Jwala)	3.4		
F <sub>1</sub> (CCA7526)	Negative			F <sub>1</sub> (CCA7526)	1.1		
<b>score</b>	<b>F<sub>2</sub></b>	<b>BC1P2</b>	<b>BC1P1</b>	<b>score</b>	<b>F<sub>2</sub></b>	<b>BC1P2</b>	<b>BC1P1</b>
Positive	24	16	10	0	20	11	10
Negative	80	29	43	1	17	9	2
				2	15	3	3
				3	8	0	3
				4	2	1	1
				5	21	9	6
Total No. of plants	104	45	53		83	33	25

\* Positive reaction means presence of the bacteria in the collar region or visual wilting symptom; Negative reaction means absence of the bacteria in the collar region and visual wilting symptom.

### Results on Phichit-1 cross

Due to low germination, low number of plants was tested in Thailand trial and conclusions should not be made with such low plant numbers. The low maximum score of the susceptible parent (2.0) indicated the low disease pressure in Thailand trial.



Thailand				AVRDC, Taiwan			
Strain		Pss71		Strain		Pss71	
Genotype	Score (24 DAI)			Genotype	Score (28 DAI)		
P1 (PBC384)	0.0			P1 (PBC384)	1.4		
P2 (Phichit)	2.5			P2 (Phichit)	5.0		
F <sub>1</sub> (CCA7527)	0.0			F <sub>1</sub> (CCA7527)	2.2		
24DAI				28DAI			
score	F <sub>2</sub>	BC1P2	BC1P1	score	F <sub>2</sub>	BC1P2	BC1P1
0	25	9	12	0	18	5	9
1	0	0	0	1	15	5	13
2	0	0	0	2	20	6	14
3	0	1	0	3	7	3	4
4	0	0	0	4	1	0	1
5	1	2	0	5	46	24	7
Total No. of plants	26	12	12		107	43	48

### Discussions

- Disease pressure of the trials conducted in China, India, and Thailand was much lower than that at AVRDC. Low disease pressure could be due to the lower virulence of the local strain or lower temperature and humidity, or other reasons.
- The frequency distribution of the progeny over disease severity did not suggest clear mode of genetic control. However, data from AVRDC trials suggested dominance of the resistance traits under different genetic backgrounds.
- It is obvious that severity of bacterial wilt can be affected by the environment easily. To overcome such a problem, it is better to use F<sub>3</sub> families and backcrosses F<sub>2</sub> families for inheritance studies. Then the experiment can be conducted with more replications and with more plants per replications and data of percentage of wilted plants can be used to determine the genetic control of the resistance trait.

### Selection for BW resistant progenies in segregating populations

#### • AVRDC

A sequential screening scheme was designed by AVRDC Pepper Unit. Summary of the 2006 bacterial wilt screening in the scheme was listed in Table 2. During the first two batches, No visual wilting symptoms were observed, even with two inoculations on the 1<sup>st</sup> batch. These could be due to the low temperature as well as the older seedling age. However, stem necrosis was observed on susceptible controls and others. Presence of *R. solanacearum* in plants showing stem necrosis was confirmed. And plants showing stem necrosis were considered susceptible and discarded. Remaining plants were transplanted to a field infested with *R. solanacearum* to further select for BW and anthracnose resistance. Whether stem necrosis is suitable selection criteria requires follow-up experiments.

Table 2. Summary of 2006 spring BW evaluation for the multiple disease selection scheme

Batch (No. of seedlings)	Code	Sowing Date	Inoculation Date for CMV/CV MV	Inoculation Date for BW (Seedling age)	Mean Temperature °C		Mean RH%		BW Incidence on PBC1367 (S)
					Max	Min.	Max	Min	
1 (1,120)	F5HP- 0605-3	26 Jan.	1 Mar.	2 Mar. and 3 Apr. (34, 49)	27.9 , 30.5	17.5 , 20.9	93.7, 93.4	57.7, 58.4	No CK
2 (5,460)	F3HP- 0603-2	26 Jan.	1 Mar.	17 Mar. (49)	29.9	20.3	93.4	53.5	20% stem necrosis (2 WAI)
	F5HP- 0605-1		3 Mar.						
	F5HP- 0605-2		1 Mar.						
3 (3,500)	F2HP- 0602-1, - 2, -3	15 Feb.	16 Mar.	21 Mar. (35)	30.5	20.9	93.4	58.4	80% wilted (3 WAI)
4 (3990)	F5HP- 0605-5	21 Feb.		28 Mar. (34)	31.7	21.4	92.8	53.5	100% wilted (3WAI)
	F6HP- 0606-3								

- Thailand*

Screening of BW to select resistant progenies was conducted in a sequential scheme to develop lines with resistance against multiple diseases. In summary, F3, F5 and F6 generations were screened for bacterial wilt along with CVMV virus. Healthy plants after screening were planted at Pichit Horticultural Center or at Center in Nakorn Phrathom province for further selection and screen for anthracnose reaction.
- India*

Screening of BW to select resistant progenies was conducted in a sequential scheme to develop lines with resistance against multiple diseases. At least 50 F5 lines showing resistance to BW and various degree of resistance to CMV, CVMV, and anthracnose were selected.
- Indonesia*

Indonesian analysis of resistance to Bacterial wilt involved two crosses between resistant parents PBC473 and TitSuper crosses with the susceptible parent PBC67 (MC5). Both parents were provided by AVRDC Bacteriology Unit as resistance source, at 98% and 93% resistant, respectively. Both cross combinations displayed high levels of dominance of resistance in the F1 generation, and high frequencies of resistant plants in the F2 generations. A complex 3-gene model of epistatic interaction was proposed, and was supported by test backcrosses to the susceptible parent. Inconsistent results were ascribed to the conclusion that the susceptible parent PBC67, was not homozygous; because of the anomalous behavior of that parent line, this judgment seems valid. The very high frequency of resistant individuals in virtually all populations of both crosses may result from the unusual susceptibility of the parent PBC67 to the local pathogen isolate, which may have been elicited by a relatively minor off-type which would normally be resistant to other pathogen isolates.

% Resistant to Bacterial Wilt (30 DAI)			
	Plants	Resistant: Susceptible	Chi-Square (3 gene model with complex epistasis)
P1 (PBC473)	20	20:0	All resistant; $\chi^2 = 0.0125$ ( $p > .9$ )
P2 (PBC67)	20	4:16	(poor fit) $\chi^2 = .6125$ ( $0.5 > p > 0.25$ )
F1	20	20:0	$\chi^2 = 0.0125$ ( $p > .9$ )
F2	200	173: 27	0.05 ( $0.9 > p > 0.75$ )
BCrF1	40	39:1	$\chi^2 = .00625$ ( $p > .9$ )
BCsF1	40	32:8	[heterozygous P2; 3R:1S] 0.30 ( $0.75 > p > 0.5$ )

% Resistant to Bacterial Wilt (30 DAI)			
	Plants	Resistant: Susceptible	Chi-Square (3 gene model with complex epistasis)
P1 (TitSuper)	23	22:1	All resistant; $\chi^2 = 0.0125$ ( $p > .9$ )
P2 (PBC67)	20	4:16	(poor fit) $\chi^2 = .6125$ ( $0.5 > p > 0.25$ )
F1	24	24: 0	$\chi^2 = 0.0125$ ( $p > .9$ )
F2	246	214 :32	0.226 ( $0.75 > p > 0.5$ )
BCrF1	40	39:1	Almost all resistant
BCsF1	40	32:8	[heterozygous P2; 3R:1S] 0.30 ( $0.75 > p > 0.5$ )

- India**  
 Resistance to Bacterial Wilt was assayed in the cross between the Indian PLV Pusa Jwala and the resistant parent PBC384. Parental, cross and backcross populations were provided by AVRDC, and screened at IHR using the local *Ralstonia solanacearum* isolate "Kerala RS". Because no symptom development was observed 20 days after infection, the plants were re-inoculated, and plants were evaluated at 3 day intervals. Final results of disease incidence evaluated 30 days after the second inoculation are presented below:

India			
	Plants	Resistant: Susceptible	Chi-Square (single dominant gene)
P1 (PBC384)	24	21:3	$\chi^2 = 0.2604$ ( $.75 > p > 0.5$ )
P2 (Pusa Jwala)	24	2:22	$\chi^2 = 0.09375$ ( $.9 > p > 0.75$ )
F1	24	20:4	$\chi^2 = 0.5104$ ( $.5 > p > 0.25$ )
F2	104	80:24	$\chi^2 = 0.1154$ ( $0.75 > p > 0.5$ )
BCrF1	48	40:8	$\chi^2 = 1.171875$ ( $0.5 > p > 0.25$ )
BCsF1	48	32:16	$\chi^2 = 4.6875$ ( $.05 > p > 0.025$ )

The occurrence of susceptible plants in virtually all generations, including the resistant parent, F1, and backcross to the resistant parent, suggests that the resistance being evaluated is not complete, and additional minor factors may be needed to establish full resistance. Resistance is not fully dominant, and selected segregates should be reevaluated, and reselected once or twice to stabilize the resistance.

#### Activity 4.2: Inheritance of CMV Resistance determined against different stains and under different environments at each country

Resistance to *Cucumber mosaic virus* was evaluated utilizing four PLV parents crossed with one of AVRDC's best sources, VC246. Seedlings of Parents, F1, F2, and backcross populations were grown under greenhouse conditions, and mechanically inoculated with a severe strain of the virus. Disease symptoms were evaluated on each plant weekly, and final data summarized on the basis of symptom development 23 days after inoculation. The tests were conducted in 2006, but results were highly inconclusive, largely because the resistant

parent failed to demonstrate its resistance in the tests. Additional seed lots were generated, and the tests repeated in 2007. Results were somewhat better, but again difficult to interpret. The PLV Phichit-1 appears to carry high levels of resistance, as confirmed in parental and segregating population performance. Pusa Jwala also displayed high frequencies of resistant plants in the segregating generations, though not in the parent sample.

#### Inheritance of Resistance to CMV in Four Susceptible backgrounds

Test 1:

CMV resistance	Pusa Jwala		Pan FLC		Jatilaba		Phichit-1	
	Resistance	Plants	Resistance	Plants	Resistance	Plants	Resistance	Plants
Pr	0.5792	33	0.6563	26	0.4778	23	0.645	30
Ps	0.682	22	0.358	21	0.714	21	0.000	22
F1	0.919	37	0.172	29	0.486	35	0.974	38
F2	0.892	158	0.286	119	0.574	122	0.986	144
BCsF1	0.853	75	0.277	47	0.507	67	1.000	70
BCrF1	0.634	71	0.393	56	0.623	61	0.909	66

Model:

X-square	-	-	-	-
Goodness of Fit	-	-	-	-
Heritability-Broad	<b>-0.602293</b>	<b>-0.135087</b>	<b>-0.062769</b>	<b>-4.255285</b>
Heritability-Narrow	-0.639947	0.379066	0.2840921	-4.956098

Test 2:

CMV resistance	Pusa Jwala		Pan FLC		Jatilaba		Phichit-1	
	Resistance	Plants	Resistance	Plants	Resistance	Plants	Resistance	Plants
Pr	0.925	40	0.688	48	0.625	48	0.831	48
Ps	0.436	39	0.292	48	0.063	48	1.000	48
F1	0.957	47	0.396	48	0.021	48	1.000	48
F2	0.932	191	0.524	191	0.313	192	0.953	192
BCsF1	0.923	91	0.367	90	0.14	86	0.969	96
BCrF1	0.914	93	0.516	93	0.542	96	0.906	96

Model:

X-square	104.01'	103.3	308.2	172.9
Goodness of Fit	-	-	-	-
Heritability-Broad	<b>-1.142002</b>	<b>0.0659402</b>	<b>0.0321012</b>	<b>-0.475942</b>
Heritability-Narrow	-0.546996	0.2723429	-0.241387	-2.213008

### **Comments:**

The results for the CMV assay are un-interpretable. The resistant parent showed greater disease incidence than some of the 'susceptible' parents; segregating populations were sometimes more resistant than either parent; backcross trends did not confirm trends found in the parents, F1 and F2 generations, heritability calculations are meaningless (they should be positive decimal values between 0 and 1). These trials will be conducted again, when fresh seed of segregating generations is produced, to see if the observed results are repeated, or if we get more comprehensible data. Inheritance patterns might be quite different from one 'susceptible' parent to another, and this needs to be confirmed through replication over time. Given the anomalous results, we will probably need to repeat these crosses and evaluations several times before we can draw statistically reliable conclusions.

### **Indonesia:**

Resistance to CMV was found to be controlled by a single recessive gene. In evaluation of heterosis and combining ability of CMV resistance and ChiVMV resistance, researchers found overdominance expressed for resistance to both pathogens in hybrid combinations. They identified two parent lines provided by AVRDC- CCA321 ("IPB C14") and PBC495 ("IPB C10")- as having strong potential for use as parents in hybrid combinations, generating resistance to both viruses in. It is not clear how the recessive gene action of CMV resistance is expressed in hybrids, unless the complementary pattern also carries resistance.

### **India:**

Resistance in a basic inheritance study displayed a pattern reflecting control by a single recessive gene. A substantial number of plants of the resistant parent developed disease symptoms, so we cannot judge it to be completely resistant to the Indian CMV virus isolate utilized. This experiment is currently being repeated, using seed obtained from AVRDC.

<b>India: CMV</b>	<b>% Resistant to Cucumber Mosaic Virus (30 DAI)</b>		
	Plants	Resistant: Susceptible	Chi-Square (Single Recessive Gene)
P1 (VC246)	18	15:3	$\chi^2=0.3472$ (0.75>p>0.5)
P2 (Pusa Jwala)	12	2:10	$\chi^2=0.1875$ (0.75>p>0.5)
F1	10	2:8	$\chi^2=0.225$ (0.75>p>0.5)
F2	119	24:95	$\chi^2=1.2353$ (0.5>p>0.25)
BCsF1	39	4:48	$\chi^2=0.2355$ (0.75>p>0.5)
BCrF1	52	13:26	$\chi^2=3.69233$ (0.1>p>0.05)

### **Thailand:**

No data reported.

### **China:**

No data reported.

### **Activity 4.3 Inheritance of Resistance to ChiVMV determined against different strains under different environments at each country**

*Chilli veinal mottle virus* (ChiVMV) has proven to be a comparatively easy disease to select for resistance under controlled screening conditions. Inheritance studies with four PLV lines as susceptible parents were conducted twice at AVRDC (see Table 3). The resistant parent (VC58a) was consistently resistant, and all PLV lines proved to be susceptible. Segregation patterns generally conformed closely to a pattern of a single dominant gene conferring resistance, as indicated by chi square estimations, with close fits in most cases. Anomalous segregation ratios (e.g. Phichit in 2006, and Pan FLC in 2007 assays) leave some uncertainty, but for practical breeding purposes, we can anticipate that good resistance can be recovered in relatively small, early generation segregating populations, and/or in conjunction with rigorous simultaneous selection for additional traits.

**Table 3. Inheritance of Resistance to ChiVMV in Four Susceptible backgrounds**

Test 1

(2006):

**CVMV**

Resistance	Pusa Jwala		Pan FLC		Jatilaba		Phichit-1	
	Resistance	Plants	Resistance	Plants	Resistance	Plants	Resistance	Plants
Pr	0.9400	35	1.0000	44	1.0000	45	1.0000	39
Ps	0.1070	28	0.0000	4	0.1760	17	0.1160	43
F1	1.0000	32	0.9470	19	0.9140	35	0.8000	40
F2	0.7320	179	0.7000	60	0.7610	142	0.6510	172
BCsF1	0.5630	87	0.4500	25	0.5610	66	0.4440	90
BCrF1	0.9520	84	1.0000	53	0.9870	75	0.9780	92

Model: Single dominant gene

X-square (6 populations)	1.816	0.8016	1.3351	11.249
Goodness of Fit	.9 >p>.75	.99 >p>.975	.9 >p>.95	0.025 >p>.05
Heritability-Broad	0.6251	0.9868	0.3312	-0.8061
Heritability-Narrow	0.0039	0.5935	0.4666	0.1358

Test 2

(2007):

**CVMV**

Resistance	Pusa Jwala		Pan FLC		Jatilaba		Phichit-1	
	Resistance	Plants	Resistance	Plants	Resistance	Plants	Resistance	Plants
Pr	0.95833	48	0.85417	48	0.97917	48	0.93750	48
Ps	0.17143	35	0.00000	48	0.02083	48	0.08333	48
F1	0.91667	48	0.63158	38	0.91667	48	0.89583	48
F2	0.71429	189	0.60417	192	0.65625	192	0.72527	182
BCsF1	0.47917	96	0.11458	96	0.41053	95	0.48958	96
BCrF1	0.86458	96	0.79365	63	0.89474	95	0.98810	84

Model: Single dominant gene

X-square (6 populations)	3.990	84.679	12.417	1.290
Goodness of Fit	.75 >p>.5	.005 >p	.05 >p>.025	.95 >p>.9
Heritability-Broad	0.3762	0.5466	0.6351	0.4656
Heritability-Narrow	-0.1820	0.0997	0.3559	0.9942
		Poor fit	Poor fit	

**Indonesia:**

From Abstract of: Xahratul Millah, 2007. Inheritance study of resistance to *Chilli veinal mottle virus* on chilli pepper (M.s. Thesis, Bogor Agricultural University)

Inheritance study for chili pepper resistance to ChiVMV infection was conducted. On this study, six generations population from the cross between PBC495 and ICPN12#4 was used. Disease incidence and score of absorbance values at 405 nm were used as variables for resistance response. Based on the experiment, it is concluded that there was no maternal effect on the

inheritance of chilli pepper resistance trait to ChiVMV infection. Resistance to ChiVMV infection was controlled by a pair of dominant major gene with full dominant gene action. Biometrical analysis to viral titer showed that resistance to ChiVMV infection was also controlled by at least a group of minor genes with gene action type followed the M[d][h][i] genetic model. Heritability values of the trait were medium to high.

It was evidenced that heterosis occurred due to interaction between genes controlling number of fruits and those controlling overdominance for fruit yield per plant, days of harvesting, resistance to CMV and resistance to ChiVMV. This gene interaction can be used for developing improved hybrid varieties. General combining ability (GCA) effect will influence specific combining ability (SCA) in which the latter is responsible for improvement of heterosis. Narrow sense heritability values of horticulture character, resistance to CMV and resistance to ChiVMV were high and narrow sense heritability values were range from low to high. Resistance to CMV and ChiVMV were controlled by each recessive and dominant gene. Two parents i.e. IPB C14 and IPB C10 can be used as donor parents for developing both CMV and ChiVMV resistance chili varieties, whereas IPB C1 can be used as donor parents for developing ChiVMV resistance chili varieties only. Three hybrids (IPB C2 x IPB C14, IPB C9 x IPB C10 and IPB C10 x IPB C1) showed resistance to both CMV and ChiVMV, 6 hybrids (IPB C2 x IPB C10, IPB C4 x IPB C10, IPB C14 x IPB C4, IPB C14 x IPB C9, IPB C14 x IPB C1 and IPB C4 x IPB C1) showed resistance only to ChiVMV.

**India:**

Seeds of lines PBC 122 (resistant parent), Pusa Jwala (susceptible parent), CCA10011, CCA 10012, CCA 9942 & CCA 9930 (F1), CCA 5365 & CCA 6667 (F2), CCA 10031 & CCA 10033 (BC1r) and CCA 10029 & CCA 10032 (BC1s) were received from AVRDC through NBPGR, New Delhi on 24<sup>th</sup> July 2008. Seeds were sown on 26<sup>th</sup> July 2008 and seedlings are inoculated by local CVMV isolate on 25<sup>th</sup> Aug 2008. Observations are to be taken..

**Thailand:**

No results submitted

**China:**

No results submitted

**Activity 4.4: Inheritance of Anthracnose resistance determined against different strains and under different environments at each country:**

Inheritance studies were conducted at AVRDC, 2005-6 by Susan Lin, and staff of Pepper unit. Anthracnose species '*Colletotrichum acutatum*' is one of the most important fruit rot diseases of pepper (*Capsicum* spp.) in Taiwan. Inheritance of resistance to anthracnose (*C. acutatum*) in pepper was evaluated in a cross between the susceptible variety 'Susan's Joy' (AVRDC 9955-15; P<sub>2</sub>) and the resistance line '0038-9155'(P<sub>1</sub>), a BC<sub>3</sub>F<sub>4</sub> selection derived from an interspecific cross between PBC 932 (*C. chinense*) and the cultivar 'IR' (PBC 535; *C. annuum*). Fruit of parents, F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub>P<sub>1</sub>F<sub>1</sub>, and BC<sub>1</sub>P<sub>2</sub>F<sub>1</sub> generations were microinjected with AVRDC's isolate Coll-153 (*C. acutatum*) at two positions on each green and each red fruits; lesion diameter was measured five days later. High level of resistance to anthracnose (*Colletotrichum acutatum*) in chilli pepper (*Capsicum* spp.) was identified in the *C. chinense* line PBC 932, and transferred into *C. annuum* through three backcrosses into the recurrent parent and the cultivar 'IR'. Inheritance of resistance was evaluated twice in a cross between this resistant BC<sub>3</sub> F<sub>4</sub> selection and the susceptible variety 'Susan's Joy'. Fruit of parents, F<sub>1</sub>, F<sub>2</sub>, BC<sub>r</sub>F<sub>1</sub>, and BC<sub>s</sub>F<sub>1</sub> generations were inoculated with the pathogen isolate Coll-153 (AVRDC) at two positions on green and red fruits. At the green fruit stage, seven of ten F<sub>1</sub> plants evaluated were resistant, but three displayed a susceptible reaction. Segregation ratio in the F<sub>2</sub> generation fit a 9R:7S model, and in the BC<sub>s</sub>F<sub>1</sub> generation of 1R:3S, suggesting control by two complementary dominant genes. However, backcross to the resistant parent (BC<sub>r</sub>F<sub>1</sub>) failed to fit the predicted 1R:0S ratio. At the red fruit stage, seven of ten F<sub>1</sub> plants proved susceptible. The ratio of resistant to susceptible plants in the F<sub>2</sub> generation approximated a 7R:9S ratio, and almost all the BC<sub>s</sub>F<sub>1</sub> progeny were

susceptible (4R:96S plants), suggesting that the resistance may be controlled by duplicate recessive genes. However, the BCrF<sub>1</sub> generation (84R:15S) did not fit the expected 1R:1S ratio. Correlation of resistance in plants as measured in green fruit vs. red fruit was 0.07, 0.17 and – 0.04, in the F<sub>2</sub>, BCrF<sub>1</sub> and BCsF<sub>1</sub> generation samples, respectively. Thus, the gene pair conferring resistance at the immature green fruit stage appears to be distinct, and independent of the pair of genes controlling resistance in mature red fruit.

**Inheritance of Anthracnose Resistance evaluated in Indonesia:** Abstract from Syukur, (2007) Inheritance study of Chili Resistance to Anthracnose Caused by *Colletotrichum acutatum*.

Anthracnose is the most prominent chili disease in Indonesia. *Colletotrichum acutatum* has been reported as a predominant species chili disease, in Asia, including Indonesia. The objective of this research is to study the genetic control of inheritance, of chili (*Capsicum annuum* L.) resistance to anthracnose caused by *C. acutatum*. The population set is developed from the crossing of resistant parent (C-15) [=AVRDC line '0209-4' the BC3 selection used as resistant parent in its inheritance studies] and susceptible parents (C-2 [PSPTC2] and C-19 ['Randu', a commercially available Open pollinated variety]). Twenty well-matured-green chili from each plant were inoculated with PYK 04 and BGR 027 isolates of *C. acutatum*. The disease incidences were observed in 5 days after inoculation. The result of the study showed that resistance to anthracnose caused by *C. acutatum* is controlled by multiple genes without maternal effect. The resistance controlling genes are recessive genes. The dominance level is categorized as partial-recessive. The action of resistance controlling genes to anthracnose was additive and dominant, where additive variance is bigger than dominant variance. Broad sense heritability value was high, whereas narrow sense heritability value was moderate. The selection for hybridization of superior chili- *C. acutatum* resistant, is better to be performed in further generation, for example by using bulk or single seed descent (SSD) method.

#### **Activity 4.5: Inheritance of Phytophthora resistance determined against different strains and under different environments at each country**

##### **AVRDC:**

Four important sources of resistance have been recognized and used at AVRDC. Dominance of resistance has been tested in F<sub>1</sub> hybrids of crosses between the eight nominated PLVs and all four resistant sources (Randomized complete block design, two replications). As shown in Table 4, most combinations display strong partial to complete dominance for expression of resistance. PLV parents generated distinct resistance percentages across the array of resistant parents, suggesting both that various genetic factors are involved in development of resistance, and also that both resistant and susceptible parents vary in the complement of resistance factors that they may carry. For example, PLVs G4, KR-B, and WR-1 tend to have partial dominance, while PLVs Phichit-1, Jatilaba, and Pusa Jwala display nearly total dominance in crosses with all four resistant parents. Anomalous results were found in specific crosses (e.g. PanFLC x PI188478, and WR-1 x PI201234) were entirely susceptible. These results reinforce the impression that several genetic factors may contribute to resistance to Phytophthora, and that both resistant and susceptible parents may contribute complementary factors. Resistant parents CM334 and PI 201234 generated the greatest frequency of complete dominance for resistance, while PI 201238, and PI 188478 are somewhat inferior in conferring hybrid dominance. PI 201238, nevertheless, is a highly promising parent for breeding programs, inasmuch as the fruit type and yield potential are closer to commercial targets than the other tested lines, and may yield more horticulturally acceptable selections. Such distinctions suggest that progress can be made in gathering together more minor or quantitative factors affecting resistance into improved inbred lines, and also that performance in hybrid combinations may depend greatly on 'combining ability', both general and specific.

Table 4: Resistance to *Phytophthora capsici* in F<sub>1</sub> hybrids of crosses between four resistant parents, and eight susceptible PLV parents

% resistant plants 3 weeks after inoculation with PC3 in F <sub>1</sub> generation cross combinations (2008)					
	Resistance source Parent (% res)	100	83	100	88
PLV (% res)		CM334	PI188478	PI201234	PI201238
0	G-4 (res CVMV)	67	9	67	78
0	PanFLC	58	0	92	71
0	Phichit-1	100	96	100	100
0	Jatilaba	100	100	100	88
8	Pusa Jwala (Res BW)	100	92	100	96
5	KR-B (res BW)	92	15	69	64
9	TitSuper	100	72	83	100
0	WR-1	83	33	0	71

Figure 4. Four important sources of *Phytophthora* Resistance (scale in cm):



CM334



PI 188478



PI201234



PI 201238

Full inheritance studies were conducted on the Project PLVs, utilizing the resistant parent line PI 201234. Because of anomalous results in performance of the resistant check lines in some trials in 2005, the populations were evaluated again in 2006 (see tables below). The Phichit-1 variety, in particular, displayed complete susceptibility in 2005 trials, but high resistance in 2006 assays; 'Jatilaba' also displayed a high frequency on non symptomatic plants in 2006, and all segregating populations had very high frequencies of resistant individuals. Two other PLV parent lines were highly susceptible, and segregating populations (especially the susceptible

backcross) displayed patterns indicating control by a relatively few major genes. Nevertheless, environmental conditions under which screening is conducted may have a profound impact on the results, and they should be closely monitored to determine the root causes of differing results. In these trials, for instance, the 2005 evaluations were conducted in the fall and winter season, when days are short, greenhouse temperatures and light levels may be relatively low; the 2006 trials were conducted during the summer season, when temperatures and humidity are relatively higher.

Table 5. Results of Phytophthora Resistance Inheritance study (AVRDC), 2005

	% Resistant PLV= Jatilaba	Chi-Square (Single Dominant Gene)	% Resistant PLV= Phichit-1	Chi-Square (Single Dominant Gene)
P1 (PI201234)	0.95	All resistant expected	0.8205	All resistant expected
P2 (PLV)	0.00	All susceptible expected	0.00	All susceptible expected
F1	1.00	All resistant expected	0.9555	All resistant expected
F2	0.8187	$\chi^2=3.9474$ (0.05>p>0.025)	0.6936	$\chi^2=2.6377$ (0.25>p>0.10)
BCrF1	0.9833	All resistant expected	0.9487	All resistant expected
BCsF1	0.5238	$\chi^2=.02381$ (0.9>p>0.75)	0.4537	$\chi^2=0.8404$ (0.5>p>0.25)

Table 6. Results of Phytophthora Resistance Inheritance study (AVRDC) using PI201234 as the resistant parent, 2006

	% Resistant			
	Pan FLC	Pusa Jwala	Jatilaba	Phichit
P1 (PBC714 = PI 201234)	1.0000	1.0000	1.0000	1.0000
P2 (PLV)	0.0000	0.0000	0.5000	0.9167
F1	0.7917	0.9583	0.9167	0.8750
F2	0.7000	0.5438	0.8495	0.8559
BCrF1	0.9583	0.8333	0.9167	0.9583
BCsF1	0.1600	0.5208	0.9375	0.8750

Unusual segregation patterns in almost all tests (e.g. high frequencies of resistance in susceptible parents Jatilaba and Phichit, susceptible backcrosses almost as resistant as resistant backcrosses, etc.) suggest that the screening conditions were imperfect. Crossing blocks were re-established, and fresh seed lines were generated for retesting. This was conducted in 2008, with much more credible outcomes.

## 2008 Evaluation of inheritance of resistance to Phytophthora wilt in four susceptible parents

Results:

Phytophthora	Pusa Jwala		Pan FLC		Jatilaba		Phichit-1	
Resistance	Resistance	Plants	Resistance	Plants	Resistance	Plants	Resistance	Plants
[PI 201234 ] Pr	0.979	48	0.979	48	0.979	48	0.979	48
[PLV] Ps	0.000	48	0.000	48	0.063	48	0.000	48
F1	0.875	48	1.000	47	0.938	48	1.000	48
F2	0.488	215	0.498	211	0.656	212	0.787	216
BCsF1	0.095	95	0.147	95	0.632	95	0.462	91
BCrF1	0.958	95	.989	95	0.958	96	0.958	96
Dominance inF1	Incomplete		Incomplete		Complete		Incomplete	
Inheritance model	Duplicate Recessive Epistasis		Duplicate Recessive Epistasis		Single Dominant		Single Dominant	
Chi-square (F2 Ratio)	9:7 4.5042		9:7 3.3492		3:1 9.5660		3:1 1.3889	
Goodness of Fit	<b>.025&gt;p&gt;.01</b>		<b>.025&gt;p&gt;.01</b>		<b>.025&gt;p&gt;.01</b>		<b>.1&gt;p&gt;.05</b>	
Chi-Square (BC1s ratio)	1:3 11.4000		1:3 4.8035		1:1 6.0632		1:1 0.3956	
Goodness of fit	<b>.005&gt;P</b>		<b>.05&gt;p&gt;.025</b>		<b>.025&gt;p&gt;.01</b>		<b>.75&gt;p&gt;.50</b>	
Heritability-Broad	0.4256		0.862		0.7327		0.9030	
Heritability-Narrow	1.2041		0.857		0.1111		-0.4835	

Chi Square tests for goodness of fit provide rather weak interpretation of the results of segregation in the several generations. Aggregated  $X^2$  assays are not possible, inasmuch as several populations (e.g. resistant parent,  $F_1$ , and resistant backcross populations) have expected frequencies of susceptible individuals of zero, making the calculated fit estimate meaningless (dividing by zero). Tentative interpretation lies in the  $F_2$  generation, with supporting confirmation in the susceptible backcross.  $F_2$  generation segregation ratios continue to provide poor fits to any simple inheritance model.  $F_1$  and resistant backcrosses all generally reflect strong dominant expression of resistance, but  $F_2$  and susceptible backcrosses fit single dominant (3R:1S) or recessive epistasis (9R:7S in the  $F_2$ ) segregation ratios. The cross between PI 201234 and Phichit comes closest to fitting a single dominant genetic model. Jatilaba X PI 201234 also approximates this pattern, but displays insufficient resistant plants in the  $F_2$ , and too many in the susceptible backcross. The remaining crosses provide a poor fit to a recessive epistatic pattern, but better than other candidate ratios. As has been shown in the array of  $F_1$  hybrid combinations, both resistant and susceptible parents may harbor minor genetic factors that exert significant impact on the expression of resistance.

Heritability calculations also provide poor results, with narrow sense heritability values greater than 1 in some cases, and negative in others. In general, however, the impression is of moderate to high broad sense heritability (indicating little environmental effect on these results), and indeterminate narrow sense heritability (indicating possible epistatic as well as additive genetic interactions). Since our experience in prior inheritance studies has taught us that environmental conditions can have dramatic, and inconsistent, impact on the expression of resistance, we are content to surmise that this particular experiment enjoyed stable and conducive conditions for disease development and/or expression of genetic resistance.

### Phytophthora resistance evaluation in India:

Earlier screens for phytophthora resistance failed to effectively differentiate between resistant and susceptible parents, presumably due to a weak pathogen isolate, low concentration of inoculum, or non-conducive environmental conditions. Repeat Tests were performed in 2007,

utilizing populations from AVRDC, utilizing a severe strain of *P. capsici*. Backcross results are highly incongruous with behavior of parents, F<sub>1</sub>, and even the F<sub>2</sub> population. Consequently, the study was repeated in 2008. In this trial, four distinct sources of phytophthora resistance were crossed with the susceptible Pusa Jwala, and tests included parents F<sub>1</sub>, F<sub>2</sub> and two backcross populations. Results recorded 10 days after inoculation with a severe local strain of *P. capsici* suggest control by a single dominant gene in four out of five cases, and a 2-gene complementary dominant factor pattern in one (cross between PI201238 and Pusa Jwala). Discrepancies were found in several populations (e.g. resistant backcrosses in PI201238/PusaJwala, and F<sub>2</sub> in PI201234/PusaJwala). On balance, however, all crosses indicated a clear bimodal segregation, with incomplete dominance for resistance in the hybrid F<sub>1</sub>, and good backcross fits. In all susceptible backcrosses, less than half of the tested plants were resistant; since 1:1 is the predicted ratio in a single dominant gene model, observed values should fluctuate around (both above and below) that mean ratio. In many cases, the ratios are closer to the 1:3 model, which would indicate a 2-gene dominant epistasis model, as expressed in the cross with the resistant parent PI 201238. The influence of PI201238 on resistance seems to be substantially weaker than other source parents: with unusually high frequencies of susceptible plants in the F<sub>1</sub>, F<sub>2</sub>, and both backcross generations. The simple Chi-square analysis conducted in this evaluation is not strong enough to generate a detailed model involving several genes with intermediate or additive effect.

	% Resistant to <i>Phytophthora capsici</i> , race 3 (12 DAI)		
<b>India: Phytophthora</b>	Plants	Resistant: Susceptible	Chi-Square (Single Dominant Gene)
P1 (CM334)	49	48:1	$\chi^2=0.00511$ (p>.9)
P2 (Pusa Jwala)	33	0:33	$\chi^2=0.0076$ (p>.9)
F1	38	35:3	$\chi^2=0.1644$ (0.75>p>0.5)
F2	65	44:21	$\chi^2=1.482$ (0.25>p>0.1)
BCrF1	86	84:2	$\chi^2=0.0261$ (0.9>p>0.75)
BCsF1	43	18:25	$\chi^2=0.8372$ (.5>p>0.25)

	% Resistant to <i>Phytophthora capsici</i> , race 3 (12 DAI)		
<b>India: Phytophthora</b>	Plants	Resistant: Susceptible	Chi-Square (Single Dominant Gene)
P1 (PI188478)	48	44:4	$\chi^2=0.2552$ (.75>p>0.5)
P2 (Pusa Jwala)	33	0:33	$\chi^2=0$ (p>.9)
F1	70	67:3	$\chi^2=0.0893$ (.9>p>0.75)
F2	34	28:6	$\chi^2=0.62745$ (0.5>p>0.25)
BCrF1	45	42:3	$\chi^2=0.6302$ (.5>p>0.25)
BCsF1	31	10:21	$\chi^2=3.2258$ (.1>p>0.05)

	% Resistant to <i>Phytophthora capsici</i> , race 3 (12 DAI)		
<b>India: Phytophthora</b>	Plants	Resistant: Susceptible	Chi-Square (2 complementary dominant genes 9R:7S in F2)
P1 (PI201238)	45	43:2	$\chi^2=0.05$ (0.9>p>0.75)
P2 (Pusa Jwala)	33	0:33	$\chi^2=0.0076$ (0.9>p>0.25)
F1	59	52:7	$\chi^2=0.7161$ (0.5>p>0.25)
F2	49	31:18	$\chi^2=0.7156$ (0.5>p>0.25)
BCrF1 & recip.	83	63:20	$\chi^2=4.581325301$ (0.05>p>0.025)
BCsF1	42	2:40	$\chi^2=8.127$ (.005>p)

% Resistant to <i>Phytophthora capsici</i> , race 3 (12 DAI)			
India: <b>Phytophthora</b>	Plants	Resistant: Susceptible	Chi-Square (Single Dominant Gene)
P1 (PI201234)	25	25:0	$\chi^2=0.01$ (0.9>p>0.05)
P2 (Pusa Jwala)	33	0:33	$\chi^2=0.0076$ (0.9>p>0.05)
F1	48	43:5	$\chi^2=0.4219$ (0.75>p>0.5)
F2	50	44:6	$\chi^2=3.84$ (0.1>p>0.05)
BCrF1	58	58:0	$\chi^2=0.0043$ (0.9>p>0.05)
BCsF1	47	10:37	$\chi^2=14.383$ (.005>p)

### Summary Inheritance Pattern table:

Phytophthora	CM334		PI188478		PI201238		PI201234	
Resistance	Resistance	Plants	Resistance	Plants	Resistance	Plants	Resistance	Plants
[Resistant source] Pr	0.980	49	0.917	48	0.956	45	1.000	25
[Pusa Jwala] Ps	0.000	33	0.000	33	0.000	33	0.000	33
F1	0.921	38	0.957	70	0.787	61	0.896	48
F2	0.677	65	0.824	34	0.633	49	0.880	50
BCrF1	0.977	86	0.933	45	<b>0.759</b>	83	1.000	58
BCsF1	0.419	43	0.323	31	0.048	42	<b>0.213</b>	47
Dominance (F1 generation)	Incomplete		Incomplete		Incomplete		Incomplete	
Inheritance model	Single dominant		Single Dominant		2 recessive genes (7R:9S)		Single Dominant	
Chisquare (F2 Ratio)	1.482		0.627		0.716		3.840	
Goodness of Fit	0.25>p>0.1		0.5>p>0.25		0.5>p>0.25		0.1>p>0.05	
Chisquare (BC1s Ratio)	0.837		3.226		8.127		10.298	
Goodness of Fit	0.5>p>0.25		0.1>p>0.05		p<0.0005		p<0.0005	



Figure 5. Segregating populations received from AVRDC are being evaluated for *Phytophthora* resistance under screen house conditions in India, 2008

## Phytophthora resistance evaluation in Indonesia:

A master's degree candidate studied the inheritance of resistance to Phytophthora using different parents than those used in other studies reported here (Yunianto, 2007). 'Cilibangi-2' is a selection from a local landrace in Indonesia, and has proven to be highly resistant to severe strains of *P. capsici*. The susceptible parent, PSPT C-1, is a breeding line from the Bogor Agricultural University Pepper Breeding program. Reciprocal crosses indicated no maternal effect, F1 populations indicated incomplete dominance for resistance, and segregating generations indicated a highly heritably control by few genes, producing strongly bimodal distributions. Segregation ratios suggest a fairly poor fit to a 2-gene model of duplicate recessive epistasis. Incomplete dominance may cause a shift of ratios in segregating populations to generate unexpectedly high frequencies of susceptible plants.

Indonesia: Phytophthora	% Resistant to <i>Phytophthora capsici</i> , race 3 (12 DAI)		
	Plants	Resistant: Susceptible	Chi-Square (Duplicate recessive epistasis F2 9R:7S ratio)
P1 (Cilibangi-2)		11:1???	All resistant expected
P2 (PSPT C-1)		0:all???	All susceptible expected
F1		43:5	All resistant expected
F2	216	122:94	$\chi^2=3.84$ (0.1>p>0.05)
BCrF1		????	All Resistant expected
BCsF1	24	6:18	$\chi^2=14.383$ (.p<0.005)

## Annex 3: Multi-disease resistant progenies selected

### Output 5: Selection of multi-disease resistant progenies completed and advanced by classical methods, complemented by molecular marker and disease diagnostics

All national partners in this project have worked together to generate candidate genotypes with high potential for adoption within their respective target production regions. Specifications of selected advanced lines developed in each country are presented in Table 1 of Annex 1. Summary comments on national program progress follow:

#### Activity 5.1: Combination of multiple disease resistance genes using conventional breeding, molecular markers and improved diagnostics for selection

**China:** While India, Thailand, and Indonesia continue to emphasize open pollinated varieties, China has focused on development of candidate hybrid varieties. A selected advanced line from this projects activities, a candidate hybrid utilizing it and a Chinese inbred parent, and seed production cages being managed in the Hainan Island winter nursery are illustrated in figures 1-4.



**Figure 1** Hybrid variety using parent selection from CCA4757

**Figure 2** Selected Parent [CCA4757 sel] (L), Local parent (r), and candidate hybrid variety (b)



**Figure 3** Seed increase cages in Winter Nursery, Hainan Island, China

**Figure 4** Selected line from CCA 4757, selected by Hunan Plant Protection Institute breeders

Table 2.a. Disease resistance in Chinese candidate lines:

<u>Line</u>	<u>Pedigree</u>	<u>Percent Resistant Plants</u>					
		<u>CMV</u>	<u>ChiVMV</u>	<u>Bacterial Wilt</u>	<u>Anthracnose</u>	<u>Phytophthora</u>	<u>Gemini virus</u>
C001	Pan-family large chilli selection X Huangxin early maturing chilli	46	40	76	10	36	not tested
C002	West River Early chilli selection X Huangxin early maturing chilli	44	36	80	16	40	not tested
C003	Pan-family large chilli Selection X Fu Dijian chilli	38	40	40	14	44	not tested
C004	Xiangxi early chilli selection X Zhu Dachang chilli	42	42	44	30	38	not tested
C005	8911 chilli selection X Jijiao early chilli Selection	50	44	60	12	32	not tested

## Project related varieties released in China by the Hunan Plant Protection Research Institute.



**C001** is a hybrid variety with medium maturity, disease resistance and high yield. Flowering begins at the ninth to eleventh node, producing horn-shaped, light green, glossy fruit 23.4 cm in length and 2.9 cm in width.



**C002** is a pickled chili with the character of medium maturity. Flowering begins at the tenth to eleventh node, producing rough horn-shaped fruit 15.8 cm in length and 5.0 cm in width, weighing about 80g. The green fruit mature to a vivid red pepper pericarp and a high pungency. The variety displays better resistance to virus diseases, and tolerates cool weather in early open field planting.



**C003** is a hybrid variety with early maturity, compact habit, continuous set, and yields up to 1500kg/667m<sup>2</sup>. Mature fruit are hot, straight and smooth, and carmine in color, measuring 14.8 cm long by 1.7 cm wide, and averaging about 10 g per fruit. It is suitable for fresh consumption, pickling, or drying. The plant is resistance to virus diseases, anthracnose, and bacterial blight; and it is drought tolerant.



**C004** is an early-to-mid-maturity, hot pepper variety. The compact plant branches heavily, with thick nodes and short internodes. Flowering begins at node 12-15, and earliest fruit may be harvested about 23 days after the beginning of flowering. The harvest period may extend as much as 150 days, producing yields of about 3000kg/667 m<sup>2</sup>. The variety is resistant to virus, anthracnose, and bacterial spot (*Xanthomonas campestris* pv. *Vesicatoria*), and is tolerant to high temperatures and sunburn. The fruit is long and straight, with a bright, smooth surface, small seed ball, and thick flesh. Individual fruit are 17.8cm long, 2.8cm wide, and weigh 34.6g to as much as 50g. It has a good flavor, and may be eaten fresh or pickled.



**C005** is a later maturing hybrid hot pepper with a compact growth habit, multiple branching, and short internodes. Flowering begins at node 13-16, and harvest may begin 30 days after commencement of flowering. Fruit are horn shaped, straight and thick fleshed, 14.0cm long by 3.2cm wide, and weighing 42g, (up to 60g). Yielding about 4000kg/667m<sup>2</sup>, the variety is resistant to virus, anthracnose and bacterial spot (*Xanthomonas*

**India:** Eighty advanced selections (48 at F<sub>7</sub>, others at F<sub>4</sub> to F<sub>6</sub> generations) from CCA crosses (made at AVRDC, and sent at F<sub>3</sub> and F<sub>4</sub> generation to India for further selection and advance) were evaluated in field growouts. Twelve lines displayed yields of at least 100 g/plant (>3 ton/ha). Twelve individual plant selections were made for advance and broader evaluation. 30 additional lines selected from crosses made in India, with non-project sources of resistance, and generated six individual plant selections. Retained resistance to Bacterial Wilt and CMV was evaluated in 20 selections. Wilt resistance (no symptoms expressed) ranged from 63 to

100% (average 88.7 +/- 8.7%); CMV resistance (no symptoms expressed) ranged from 36 to 100% (average 84.3 +/- 15.2%). One selection displayed 100% resistance to both BW and CMV, and two others were completely resistant to CMV (Indian isolate B1C), and 90% or more resistant to BW. Data regarding resistance to ChiVMV or Anthracnose not presented.

S. No.	Lines	Pedigree	Days to 50% flowering	Days to 50% fruit ripening	Fruit length (cm)	Fruit width (cm)	Dry fruit yield/pt (g)	Plant height (cm)	Plant spread (cm)	Resistance reaction to diseases (%)			
										CMV resistance	ChiVMV resistance	BWR	Anthracnose fruit rot incidence (lesion size in mm)
1	F7 PJDH 14	PJ/EG132//AR4/PMR76	35.33	100.67	10.00	1.00	134.83	76.00	52.17	86	71	90	7 (3.1)
2	F8 PJDH 50	PJ/PSB//AR4/PMR76	44.00	98.33	8.67	0.80	125.32	91.67	56.50	62	89	80	40 (3.3)
3	F8 CCA 5853	PJ/ICPN 12#4	43.00	110.00	9.33	1.07	126.77	95.00	61.00	17	7	91	25 (3.4)
4	Pusa Jwala	PLV	51.67	114.33	10.67	0.87	50.47	76.67	48.00	22	24	38	0 (20.0)
5	F7 CCA 5492	G4/0209-4//G4/PBC495	56.00	116.00	7.33	0.80	124.50	78.33	57.33	29	11	75	51 (1.3)
6	F7 CCA 5478	G4/PBC122//G4/0209-4	42.33	105.67	8.00	0.93	142.34	88.00	53.83	30	19	95	20 (3.3)
7	F8 CCA 5479	G4/VC246//G4/0209-4	38.00	104.00	8.67	0.93	151.68	94.00	67.83	35	41	93	41 (6.0)
8	F8 CCA 5488	G4/0209-4//G4/VC246	56.00	112.00	7.33	0.87	118.27	95.67	63.00	67	50	82	12 (4.8)
9	F8 GDH 57	G4/97-7125-2//AR4/PMR76	47.33	111.67	9.33	0.80	162.58	71.67	58.67	67	76	93	41 (5.0)
10	G4	PLV	48.00	107.67	5.33	0.73	63.37	98.00	56.00	18	20	22	0 (10)
	SEm		4.42	2.97	0.81	0.07	3.31	8.54	4.23	-	-	-	-
	CD@5%		13.13	8.82	2.41	0.22	9.83	25.37	12.57	-	-	-	-
	CD@1%		17.99	12.09	3.30	0.30	13.47	34.75	17.21	-	-	-	-
	CV%		13.51	3.89	13.54	11.89	2.83	13.96	10.41	-	-	-	-

The above mentioned promising lines were planted in replicated trial in June-July 2008, and are at flowering stage. In another trial they are also being evaluated using GAPs *i.e.* with drip irrigation and polyethylene mulch cover. At fruiting stage the promising lines will be identified by the Institute Varietal Committee for release. Subsequently, the most promising 2 or 3 lines will be entered in All India Coordinated Research Project (Vegetable Crops) for national release and also simultaneously will be entered in State Varietal Release Trials. Meanwhile farmers will be invited to choose the promising line from the existing Institute trial and seeds of those lines will be provided to them for multilocation trials. The farmers' field evaluation data will also be used for national as well as state level varietal release.

**Figure 5:** Advanced generation selections of multi-disease resistant chili peppers developed at IHR, Bangalore, India

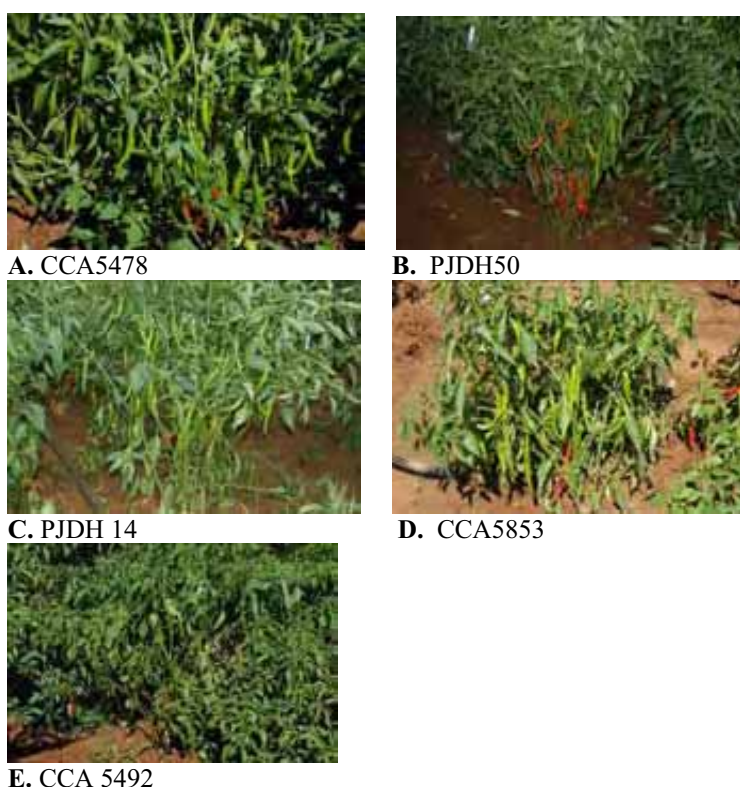


CCA5479





**Figure 6.** Advanced lines from India



**Thailand:** Despite setbacks in early phases of the project, Dr. Siri Kumphai has actively advanced early generation selections from AVRDC, and has identified two lines which are currently being multiplied, and staged for demonstration to farmers. These lines are F7 selections from the cross CCA5684, made at AVRDC in 2003, with the pedigree: SriSaket-1/0209-4//SriSaket-1/PBC495. At least partial resistance to two of the four of the project target diseases has been documented, and it is anticipated that further reselection will be conducted in cooperation with interested farmers in various production regions. Replicated (four reps) performance trials were conducted at three locations, comparing three selected lines to two local check varieties, utilizing production methods similar to those recommended by AVRDC (Raised beds approximately 1.5 meters wide, with double lines of plants spaced 35-40 cm apart; beds covered with rice straw mulch; water applied manually with sprinkler hose). Dr.

Kumphai will be retiring in a few years, but he has involved two other breeders at the Phichit station in managing the project materials, and he expects that they will continue the program in future variety development, and disease resistance screening. They continue to grow out, screen, and select progenies provided to them from AVRDC, and are crossing promising selections with locally preferred varieties, to improve fruit type.

	Transplant Date	First Harvest Date	Last Harvest Date	Number of Harvests
Pichit hort. Center, Phichit Province	12 December 2007	22 February 2007	8 May 2007	12
Srisaket Hort. Center, Srisaket Province	9 January 2007	20 March 2007	18 June 2007	15
Karnchanaburi Hort. Center, Karchanaburi Province	16 January 2007	31 March 2007	2 July 2007	13



**Figure 7.** Dr. Siripong Kumphai and two Research Associates in Project pepper advanced line evaluation trial at Phichit Station, 1/15/2008

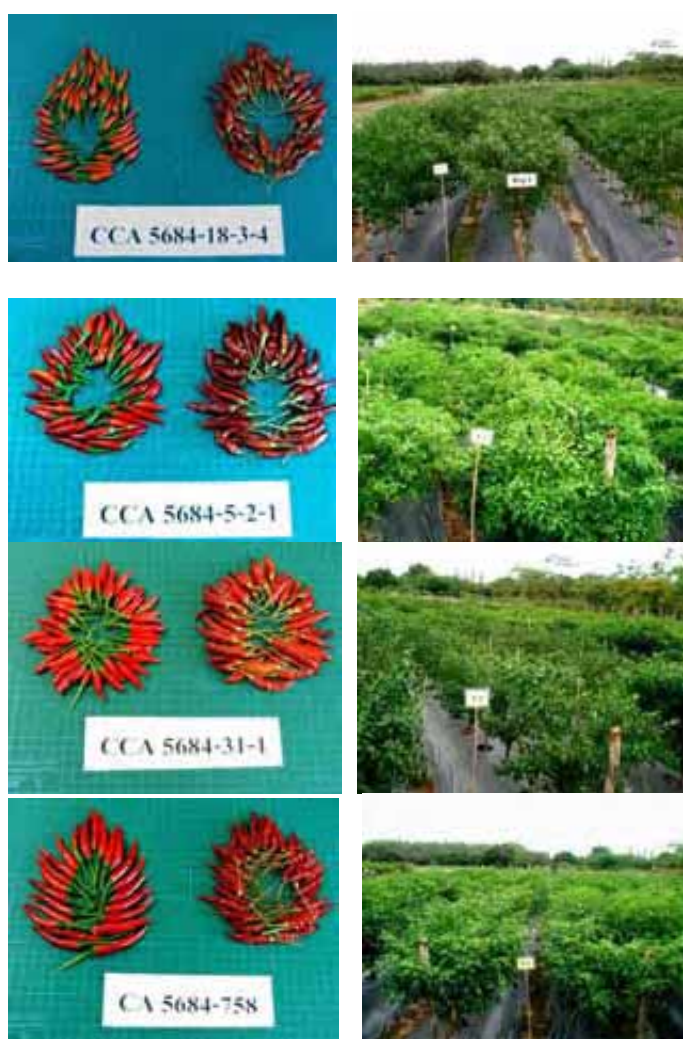


**Figure 8.** Two candidate lines in evaluation trial, Phichit Station, Thailand, 1.15/2008

Table 2.b. Performance of four candidate lines, averaged over three trials in Thailand, 2008

		CCA5684-18-3-4	CCA5684-5-2-1	CCA5684-31-1	CCA5684-75-8	Srisaket-13
Pod Characteristics						
	Peduncle length (cm)	3.41	3.17	3.09	4.72	4.71
	Pod Length (cm)	3.94	4.27	4.77	6.09	6.64
	Pod diameter (cm)	0.68	0.86	0.88	0.78	0.88
	Seed/pod	37.9	58.3	56.15	63.4	66.3
Chili Pod Yield (gram/plot)						
	Average Fresh Weight	19500	28600	18200	22350	34300
	Average Dry Weight	5450	8500	4500	6100	10050
Plant Size						
	Average Plant Height	81.25	85.38	59.1	100.5	87.51
	Bush spread (E-W x N-S, cm)	85.73 x 74.85	71.38 x 62.05	72.08 x 62.08	81 x 69	80.03 x 66.15
		74.85	62.05	62.08	69	66.15
Flowering Characteristics						
	First Flower (DAP)	31	28	32	34	28
	50% Flowering (DAT)	34	32	37	37	32
	100% flowering (DAT)	41	38	42	41	38

These lines have been multiplied, and are being distributed to farmers as they requested following field demonstrations. Most of farmers expressed interest in some of lines and have asked for seed to plant in their fields.



**Indonesia:** At least four advanced lines have been selected in Indonesia for evaluation at the farmer level, and potential release. Cross combinations were made in Indonesia, utilizing disease resistant lines from AVRDC (ICPN12#4, PBC495, ICPN7#3, CCA321, and 0209-4) and selected parents from the Indonesian breeding program (IPB C2, and IPB C). Farmer evaluations have not yet been completed.

(ICPN 12#4 X PBC 495) X (IPB C2 X ICPN 7#3)
(IPB C2 X CCA 321) (ICPN 12#4 X PBC 495)
(ICPN 12#4 X IPB C4) X (ICPN 12#4 X PBC 495)
(ICPN 12#4 X IPB C4) X (PBC 495 X IPB C2)
(IPB C2 X CCA 321) X 0209-4

**AVRDC:** Advanced lines carrying multiple disease resistance in Project national backgrounds (PLVs), are being evaluated for final selection and increase at AVRDC. These lines have all been screened and reselected under managed disease screens in Taiwan, utilizing aggressive strains of the several pathogens. These lines are being made available to the appropriate national partners, to be evaluated under their conditions. Three lines incorporating resistance to several of the project target pathogens have been included in the 2008 International Chili Pepper Nursery (ICPN18), which is distributed world-wide, to cooperating researchers who will evaluate the lines under their local production environments. As of Mid September, 2008, 42 sets have been sent out from AVRDC. Ten lines, incorporating resistance to three or four of the target pathogens, are currently being tested in AVRDC's 2008 Hot Pepper Preliminary Yield Trial (08HPPYT), and several of these will be chosen for inclusion in the 2009 ICPN19 trial set. See below for more details.

Mid-generation progenies produced in Taiwan and tested in several partner countries, especially Thailand, have proven successful in displaying usable levels of multiple resistances against pathogen populations in the target environment, and reasonably good adaptation to the local climate factors. Consumer preferences in fruit traits are difficult to match in differing locations, and local screening and selection continues to be necessary for successful variety development. The large number of multi-disease resistant selections derived from crosses with Indonesian PLVs is due to several factors: The Indonesian PLVs 'Jatilaba' and 'TitSuper' carried good resistance to Bacterial Wilt, so this did not pose a selection hurdle; The lines also performed better than PLVs from other countries in incorporating some resistance to Anthracnose; these lines also display good combining ability and contribute well to economically useful yield potential and horticultural fruit type.

Resistance (generation)	Country Origin of Predominant Local Variety			
	China	India	Indonesia	Thailand
CMV, ChiVMV, BW, Anthr (F <sub>7</sub> )			8	7
CMV, ChiVMV, BW, Anthr (F <sub>6</sub> )	9		5	
CMV, ChiVMV, BW, (F <sub>6</sub> )		7	18	9
CMV, BW, Anthr (F <sub>7</sub> )			32	
ChiVMV, BW, Anthr (F <sub>7</sub> )			22	
PC3 (F <sub>6</sub> )	18	22	26	24

CMV: *Cucumber mosaic virus*; ChiVMV: *Chilli veinial mosaic virus*; PVY: *Potato Virus Y*; ToMV: *Tomato mosaic virus*; BW: *Bacterial Wilt (Ralstonia solanacearum)*; Anthr: *Anthracnose (Colletotrichum acutatum)*; PC3: *Phytophthora, race 3 (Phytophthora capsici)*; WTG: whitefly transmitted geminivirus.

AVRDC-pepper breeding unit has continued to supply cooperators with seed for continuing evaluation for use in the national program breeding programs (see Table 2.c). This included advanced generation selections with resistance to three or more diseases, intermediate

generation selections carrying resistance to phytophthora blight, and germplasm and early generation progenies with potential resistance to whitefly transmitted geminiviruses. Shipment to some countries (notably India and Indonesia) has been hampered by cumbersome Import Permit requirements, and related delays in transit.

Table 2.c. Shipments of pepper seed from AVRDC to Project Cooperators 2005-2008.

Country	Year	Entries	Nature
China	2005	97	GTZ-F1,F2,F3,F4
China	2007	16	GTZ-CMV,ChiVMV inheritance study lines
China	2007	25	Advanced generation multi-resistant selections, WTG resistant (?) progenies
China	2008	11	Advanced generation multi-resistant selections, phytophthora resistant selections
<b>China -Total</b>		<b>149</b>	
India	2005	93	GTZ-F1,F2,F3,F4
India	2005	52	GTZ-F1
India	2008	34	ICPN16,17&ISP6,7&misc.
India	2008	64	CMV,ChiVMV,PC inheritance, anthracnose resistant lines
<b>India- Total</b>		<b>243</b>	
Indonesia	2005	314	GTZ-F1,F2,F3,F4
Indonesia	2006	6	Disease Resistant parents
Indonesia	2007	114	Advanced generation multi-resistant selections; lines to screen for WTG resistance
Indonesia	2007	16	Advance generation multi-resistant selections
Indonesia	2008	82*	Advanced generation multi-resistant selections
Indonesia	2008	159*	Advanced generation multi-resistant selections, phytophthora resistant selections, WTG resistant (?) progenies
<b>Indonesia- Total</b>		<b>691</b>	
Thailand	2005	188	GTZ-F1,F2,F3,F4
Thailand	2006	24	F5 selections, WTG resistant candidate lines
Thailand	2007	59	WTG resistant (?) progenies
<b>Thailand - Total</b>		<b>271</b>	

\*Includes entries targeting tasks for the related ACIAR-funded project on integrated disease management to control pepper fungal diseases (Phytophthora and Anthracnose) and whitefly transmitted geminivirus

#### AVRDC Advanced lines generated and tested in 2008 ICPN18

Index	Code	Code Pedigree	Name Pedigree	Resistance
ICPN18-02	CCA5850-12-5-1-1-5	PBC1571/9852-173	KR-B//Kulim/HDA 295	Anthr, BW,ToMV
ICPN18-05	CCA5829-2-2-bk-2-2	PBC374/9950-5197	Jatilaba//Kulim/HDA248	CMV, ChiVMV, BW
ICPN18-06	CCA5835-3-2-bk-1-1	PBC376/9950-5197	Tit-Super//Kulim/HDA248	CMV, ChiVMV, BW



KR-B/Berke's Joy



Jatilaba/CCA321



Tit Super/CCA321



#### Spring 2008 ICPN18 extracted performance data

Entry	Code	Fruit l x w (cm)	Fruit Weight (g)	Total Yield (t/ha)	Subjective Scores (0= Bad, 5=very good)				
					Vigor	Disease	Acceptance	Yield	Overall
ICPN18-2	KR-B//Kulim/HDA 295	11.9 x 0.9	4.8	7.0	3.5	3.3	3.7	3.5	3.0
ICPN18-5	Jatilaba//Kulim/HDA248	10 x 1.7	8.3	7.2	3.8	3.0	2.7	3.3	3.0
ICPN18-6	Tit-Super//Kulim/HDA248	10.0 x 1.7	9.1	9.1	4.0	3.8	2.7	3.3	3.0
ICPN18-4	Kulim/HDA 295 (check)	8.9 x 1.6	8.5	9.5	3.3	3.2	3.8	3.7	3.8

These three lines are currently available to all researchers to evaluate in their locations, and to adopt for commercial multiplication and distribution, for further reselection, or as parents in crosses with locally adapted lines.

#### Advanced lines Tested in 2008 Hot Pepper Preliminary Yield Trial (08HPPYT)

These ten lines were generated with 3-way crosses and selection for resistance to one or more diseases in almost every segregating generation. The methodology proposed in this project, of screening for two to three resistances in the same generation, has been applied, and generated these lines. Early and mid generation populations of these crosses had been shared with project partners, and some generated useful selections in the target countries as well as in Taiwan. The Cross CCA5684, for example, was the original source of three selections being prepared for release in Thailand.

Entry	Source	Pedigree	Parentage	Resistances
08HPPYT-07	CCA5524-28-3-2-1-2	CCA5340/CCA5342	Jatilaba/0209-4//Jatilaba/PBC495	ChiVMV, CMV, BW, Anth.
08HPPYT-08	CCA5620-06-1-1-1-1	CCA5384/CCA5392	PHT-1/0209-4//PHT-1/PBC495	ChiVMV, CMV, BW, Anth.
08HPPYT-09	CCA5684-05-1-2-1-1	CCA5395/CCA5403	SSK-1/0209-4//SSK-1/PBC495	ChiVMV, CMV, BW, Anth.
08HPPYT-14	CCA5607-6-2-1-1-2-1	CCA5393/CCA5384	PHT-1/VC246//PHT-1/0209-4	CMV, PVY, BW, Anthr
08HPPYT-15	CCA5607-6-2-1-1-2-2	CCA5393/CCA5384	PHT-1/VC246//PHT-1/0209-4	CMV, PVY, BW, Anthr
08HPPYT -18	CCA5515-1-1-1-1-3-2	CCA5340/CCA5350	Jatilaba/0209-4//Jatilaba/PBC122	ChiVMV, PVY, BW, Anthr
08HPPYT-19	CCA5515-1-1-1-1-4-4	CCA5340/CCA5350	Jatilaba/0209-4//Jatilaba/PBC122	ChiVMV, PVY, BW, Anthr

Entry	Source	Pedigree	Parentage	Resistances
08HPPYT-20	CCA5515-1-1-1-1-5-1	CCA5340/CCA5350	Jatilaba/0209-4//Jatilaba/PBC122	ChiVMV, PVY, BW, Anthr
08HPPYT-21	CCA5515-1-1-2-1-2-2	CCA5340/CCA5350	Jatilaba/0209-4//Jatilaba/PBC122	ChiVMV, PVY, BW, Anthr
08HPPYT-22	CCA5515-1-1-3-1-6-1	CCA5340/CCA5350	Jatilaba/0209-4//Jatilaba/PBC122	ChiVMV, PVY, BW, Anthr

### 08HPPYT Entries



08HPPYT-07



08HPPYT-08



08 HPPYT-09



08HPPYT14



08HPPYT15



08HPPYT18



08HPPYT 19



08HPPYT20



08HPPYT21



08HPPYT 22

Spring 2008 Hot Pepper Preliminary Yield Trial extracted performance data

Entry	Name	Fruit	Fruit	Total	Subjective Score (1-Bad, 5-Very Good)				
		L x W (cm)	Weight (g)	Yield (t/ha)	Vigor	Disease	Acceptance	Yield	Overall
08HPPYT-7	Jatilaba/0209-4//Jatilaba/PBC495	9.4 x 1.7	8.9	3.7	3.3	3.0	3.0	2.5	2.8
08HPPYT-8	PHT-1/0209-4//PHT-1/PBC495	8.9 x 1.1	4.0	5.2	3.5	3.8	3.0	3.3	3.0
08HPPYT-9	SSK-1/0209-4//SSK-1/PBC495	6 x 1.1	3.2	3.7	4.5	4.5	4.0	3.3	3.8
08HPPYT-14	PHT-1/VC246//PHT-1/0209-4	13.0 x 1.5	8.0	2.0	4.8	4.8	2.5	2.3	2.8
08HPPYT-15	PHT-1/VC246//PHT-1/0209-4	12.6 x 1.5	7.6	0.7	5.0	4.5	2.5	2.0	2.5
08HPPYT-18	Jatilaba/0209-4//Jatilaba/PBC122	10.2 x 1.2	6.5	6.0	4.5	4.0	3.3	3.3	3.3
08HPPYT-19	Jatilaba/0209-4//Jatilaba/PBC122	10.3 x 1.2	6.2	4.7	4.0	4.0	3.3	3.3	3.3
08HPPYT-20	Jatilaba/0209-4//Jatilaba/PBC122	10.9 x 1.3	6.6	6.0	4.3	4.3	3.3	3.3	3.3
08HPPYT-21	Jatilaba/0209-4//Jatilaba/PBC122	10.3 x 1.2	5.7	6.8	4.0	4.0	3.8	3.5	3.8
08HPPYT-22	Jatilaba/0209-4//Jatilaba/PBC122	10.4 x 1.3	6.3	2.1	3.5	3.0	3.0	3.0	3.0
08HPPYT-23	Kulim/HDA248 (check)	10.6 x 1.8	9.9	11.1	4.8	4.0	3.3	3.8	3.8

Numerous additional advanced lines continue to be reselected and evaluated for potential release. Several will be released for evaluation and utilization by interested researchers, through inclusion in future International Chili Pepper Nurseries. As repeated testing for disease resistance identify the most stable lines, they can be tested in target countries against local pathogen isolates. It may be noted that the greatest success in recovering multi-disease resistant lines came with the use of PLVs from Thailand (SriSaket-1 [SSK-1], and Phichit-1 [PHT-1]) and from Indonesia (KR-B, Tit-Super, and Jatilaba). Parental backgrounds of Chinese and Indian PLVs produced few or no Anthracnose-resistant selections, and had continuing problems with poor fruit type and yield potential under Taiwan conditions. By way of contrast, Dr. Reddy in India, had substantial success in screening and selecting for multiple resistant lines under the target local conditions of southern India, utilizing the Indian PLVs 'Pusa Jwala' and 'G-4'.

F7 generation selections made in 2006 (planting 0637), and 2007 (planting 0737)

**Cross Pedigree Selections Resistances**

**0637 F7 Entries:**

CCA5850	KR-B/ICPN12#4	5	Anthr,BW,PVY, ToMV
CCA5708	Tit-Super/VC246//Tit-Super/PBC122	5	CMV, ChiVMV, PVY
CCA5814	Jatilaba/CCA321	11	CMV,ChiVMV,BW
CCA5835	Tit-Super/CCA321	8	CMC,ChiVMV, BW
CCA5840	KR-B/ICPN17#3	7	CMV,ChiVMV,BW

**0737 F7 Entries:**

CCA5524	Jatilaba/0209-4//Jatilaba/PBC495	8	ChiVMV,CMV, BW, Anth.
CCA5620	PHT-1/0209-4//PHT-1/PBC495	6	ChiVMV,CMV, BW, Anth.
CCA5684	SSK-1/0209-4//SSK-1/PBC495	1	ChiVMV,CMV, BW, Anth.
CCA5511	Jatilaba/VC246//Jatilaba/0209-4	2	CMV, PVY, BW, Anthr
CCA5543	KR-B/VC246//KR-B/0209-4	26	CMV, PVY, BW, Anthr
CCA5607	PHT-1/VC246//PHT-1/0209-4	4	CMV, PVY, BW, Anthr
CCA5515	Jatilaba/0209-4//Jatilaba/PBC122	22	ChiVMV, PVY, BW, Anthr

CMV: *Cucumber mosaic virus*; ChiVMV: *Chili veinal mosaic virus*; PVY: *Potato virus Y*; ToMV: *Tomato mosaic virus*; BW: *Bacterial Wilt (Ralstonia solanacearum)*; Anthr: *Anthracnose (Colletotrichum acutatum)*

## Activity 5.2 Phytophthora and leafcurl virus resistant sources crossed with promising progenies carrying multiple resistance

Initially, PLVs were crossed with a source of resistance to phytophthora blight, and screened for two or more generations, using AVRDC's severe strain of the pathogen. Resistant F<sub>4</sub> selections were then crossed with advanced generation, multi-disease resistant selections to combine resistance to phytophthora blight with resistance to CMV, ChiVMV, PVY, BW, and/or Anthracnose. Segregating populations will be filtered using the multi-sequential screening protocol developed in this project.

Selection of lines combining Phytophthora resistance with resistance to CMV, ChiVMV, BW, and Anthracnose: F<sub>2</sub> Populations (0802, Spring 2008)

Cross	Code	Pedigree	Selections
CCA9615	CCA5524-07-1-1-3/CCA6806-1-2-1	Jatilaba/0209-4//Jatilaba/PBC495//Jatilaba/PI201238	5
CCA9616	CCA5524-20-1-1-1/CCA6806-1-2-1	Jatilaba/0209-4//Jatilaba/PBC495//Jatilaba/PI201238	9
CCA9620	CCA6274-3-1-1/CCA7050-1-1-1	KR-B/VC211a-3-1-1-1//KR-B/ICPN12#4//PI201238/KR-B (res BW)	14
CCA9624	CCA6313-1-1-1/CCA6570-1-1-1	Pan FLc/CCA321//CCA321//Pan FLc/PI188478	4
CCA9630	CCA6313-1-1-2/CCA7049-1-1-1	Pan FLc/CCA321//CCA321//PI201238/Pan FLc	3
CCA9632	CCA6388-1-1-1/CCA6636-2-2-1	PHT-1/ICPN12#4//PHT-1/CCA321//PHT-1/PI188478	10
CCA9636	CCA6388-1-1-2/CCA6636-2-2-1	PHT-1/ICPN12#4//PHT-1/CCA321//PHT-1/PI188478	12
CCA9648	CCA5620-06-1-2-1/CCA6636-2-2-1	PHT-1/0209-4//PHT-1/PBC495//PHT-1/PI188478	14
CCA9652	CCA5620-06-2-1-3/CCA6636-2-2-1	PHT-1/0209-4//PHT-1/PBC495//PHT-1/PI188478	9
CCA9657	CCA5620-06-2-1-4/CCA6636-2-2-1	PHT-1/0209-4//PHT-1/PBC495//PHT-1/PI188478	4

### Whitefly Transmitted Geminivirus Resistance

The variety 'Lorai' has been identified in India as carrying resistance to *Geminivirus*. Because the virus is not found in Taiwan, selections from candidate cross combinations have been selected in Taiwan only for performance, yield potential, and fruit shape and size. F<sub>5</sub> generation progenies for have been distributed to Thailand and/or Indonesia for field evaluation. Feedback regarding observed resistance to field disease pressure has not yet been received.

Cross	Pedigree	F5 Progenies
CCA7423	Lorai/Pan FLC	35
CCA7463	Pan FLC/Lorai	9