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# BIOLOGICAL CONTROL OF CUCUMBER MOSAIC VIRUS (CMV), AN IMPORTANT DISEASE CAUSING AGENT OF HORTICULTURAL CROPS IN INDONESIA


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## EXECUTIVE SUMMARY

The overall objective of the research project is to establish biological control of cucumber mosaic virus (CMV) with using a CMV satellite, namely CARNA 5 (CMV-associated RNA 5), on peppers. This research program was implemented by A.S. Duriat, Y. Sulyo, R. Sutarya and A. Muharam. DR. J.M. Kaper from USDA, Maryland, U.S. who is the Co-Principal Investigator, is the conceptual father of CMV's satellite. He provided advices and guidances all activities. Day to day activities were under the supervision of Director Lembang Horticultural Research Insitute (LEHRI), DR. A.A. Asandhi. LEHRI and a Ducth supported project (ATA-395) also contributed financial support for field tests especially after December 1990 in order to complete the trials.

All rersearch activities related to the production of vaccine, attenuated CMV, have been acomplished. Protective vaccine, mixed CMV-2 + CARNA 5, has been obtained and it gave promising capability to protect pepper plants against the infection of other CMVs, its helper virus in controlled condition. However , field trials in different pepper-growing areas should be improved and continously investigated because of the complexity of environmental factors in terms of the incidence of other aphid-transmitted viruses which commonly occurred in the field.

As the consequence of this achievement, further research has to be continued so that this biological control effort can be applied in larger scales at farmer levels. This will provide important economic justification in terms of increasing national pepper production in Indonesia. In general, the technique will strengthen the development of Integrated Pest Management (IPM) Program.

With the improvement of the Virology Section of LEHRI, the Virologists of the Institute was able to adopt advanced technology of viral satellites. This is a new capacity for developing research in biotechnology.

## RESEARCH OBJECTIVES

Cucumber mosaic virus (CMV) which causes plant disease are spread widely in various countries. The virus has an extremely wide host range; it affects some 775 plant species of 85 families (Douine et al. cit. Kaper, 1983). CMV can be transmitted by more than 60 species of aphids, especially *Aphis gossypii* and *Myzus persicae* (Kennedy et al., 1962). In addition, CMV disease is seed-borne in certain plants (Mandahar, 1978). This virus causes severe losses in many horticultural crops. The Prime example is pepper, which lack genetic sources of CMV resistance (Greenleaf, 1986). In our trial indicated that none of 49 accessions of pepper tested for resistance to CMV so far has resistant trait (Sutarya and Duriat, 1991; Duriat et.al, 1991). Because no effective viricide has been developed to control viral crop diseases, such control of CMV has thus far been directed at the aphid

vectors by which CMV is transmitted in the field. This involves the use of incecticides, oil sprays and reflective surface. Such methods are cumbersome, expensive, and could adversely affect human beings and/or beneficial insects.

This proposal, however, suggests using a novel approach towards controlling CMV disease in horticultural crops. It proposes to develop a "vaccine" which can be applied to crop plants in the seedling stage. This vaccine will consist of a combination of live CMV and its associated satellite, isolated from an indigenous source, and will be designed to produce a mild symptomless low-level infection in plants.

In laboratory experiments, several such combinations have been shown to effectively exclude new CMV infections of the vaccinated plants. This principle will be tested systematically on pepper in a larger scale, first under controlled conditions, and later under field conditions on severals location at the LEHRI.Finally, tests will be carried out in different geographically separated areas of high CMV infestation throughout Indonesia. The results will be evaluated in terms of decreased disease susceptibility of the plants, improved fruit yield and quality. If successful, the research will enable eventual dissemination of locally produced vaccines to Indonesian farmers.

## METHODS AND RESULTS

### A. Methods

Research activities were carried out in LEHRI laboratories at Lembang , Segunung Horticultural Research Station (SHRS), and some in Microbiology Laboratory of USDA Beltsville during the project period. Field trials were carried out in Lembang, Segunung (Experimental fields for highland), Kramat, Subang, Madura (Experimental fields for lowland) and Bekasi (farmer's fields).

#### a. Survey for Collecting Samples

Surveys were conducted in different geografical areas in Java, South Sumatra and Lombok. Samples, tissues of diseased plants with virus-like symptoms, were respectively collected and stored in plastic vial containing a hygroscopic material for further investigation. Symptoms on each plant were recorded.

#### b. Virus Indexing

All samples were serological tested by using enzyme-linked immunosorbent assay (ELISA) against the CMV antiserum. Numbers of samples were also tested to other viral antisera i.e. Potato virus Y (PVY), Tobacco etch virus (TEV), Tobacco

mosaic virus (TMV), Alfalfa mosaic virus (AMV) and Chilli veinal mottle virus (CVMV).

### c. Bioassay

Test plants used in this activities were : *Browwalia cordata*, *Capsicum annuum*, *Chenopodium album*, *C.amaranticolor*, *C. murale*, *Cucumis sativus*, *Cucurbita pepo*, *Datura stramonium*, *Gomphrena globosa*, *Lycopersicon esculentum*, *Nicandra physaloides*, *Nicotiana glauca*, *N. glutinosa*, *N. tabacum* Havana 423, *N. tabacum* White Burley, *N.tabacum* Xanthi nc, *Physalis angulata*, *P. floridana*, and *Solanum melongena*.
Inoculation was conducted by using a mechanical method.

### d. CMV Isolation and Determination of Its Strains

Samples which positive to CMV were additional by treated with consecutive inoculation through certain indicator plants to differentiate symptoms. The indicator plants used for isolating CMV were *Cucurbita pepo* (Zucchini) and *N. tabacum* Xanthi nc.

### e. CMV Purification

Purification method used was the method described by Lot and Kaper (1976), utilizing polyethylene glycol and differential ultracentrifugation. Quality of the virus preparation and the viral concentration was determined using spectrophotometry and calculated by this formula :

$$\text{Virus Cons.} = \frac{\text{Abs.at 260 nm} \times \text{Dilution factor.}}{5\ \text{(extinction coef.)}}\ \text{mg/ml}$$

### f. RNA Extraction

CMV RNAS were isolated by means of phenol extraction of the purified virus in solution of 0.02 M Na-phosphate pH 7.0 and 1 percent Sodium dodecyl sulphate (SDS) followed by two cycles of ethanol precipitation. The following formula was used to determine the RNA concentration

$$\text{RNA Cons.} = \frac{\text{Abs. at 260 nm} \times \text{Dilution Factor}}{25\ \text{(extinction coef.)}}$$

### g. Characterization of RNAs

Characterization of RNAs was carried out by electrophoresis of total RNA or degraded virus on a 2% composite

polyacrylamide-agarose or "survey gel". Gel was run at 80-90 Volt about 3-4 hours, just after the blue color of Bromophenol Blue (BPB) reached the bottom of the cell. This gel separated all genomic CMV RNA components and also showed the present of CARNA 5.

### h. Separation of Viral RNAs and CARNA 5

Total CMV RNA was loaded on sucrose gradient ultracentrifugation. By means of top fractionation CARNA 5 is separated from RNA 4 and RNA 123. According to the size each RNA was separated in different layer on sucrose in gradient. The fractionator read a different peak of each RNA. By collecting the peak and subsequent ethanol precipitation and resuspension , each RNA fraction was recovered.

### i. Stimulation of Satellite RNA Development on Tobacco Plants

CMV isolates were maintained on tobacco (Xanthi nc). Every one or two week after inoculation (showing symptoms) they were reinoculated to the new healthy tobacco seedlings. The occurrence of satellite (CARNA 5) on infected plants was monitored by using total nucleic acid analysis (TNA) of White and Kaper (1988). Symptoms were also observed.

### j. Introduction of S-CARNA 5 into Indonesia CMV isolates

Tomato Rutgers in two true leaves stage were inoculated by a mechanical method with CMV-4 (mild) and CMV-2 (severe). Each treatment consisted of 20 plants. Two days later, 10 plants of each were reinoculated with 5 ug/ml of S-CARNA 5 in 0.03 M phosphate buffer. Occurrence of satellite RNA on infected plants was analyzed by using the total nucleic acid analysis of White and Kaper (1988) at 1, 2 and 3 weeks after inoculation of satellite.

### k. Evaluation of Candidate Vaccine Under Controlled Condition

Evaluation of the efficacy of candidate vaccines on peppers has been done by using 20 ug/ml purified vaccine (virus plus satellite) or 1/10 sap of infected tissues in inoculation buffer. Symptoms developed on the vaccinated plants which were challenged with or without severe strains were observed.

Experiments were carried out in a glasshouse using pots with sterilized soil or in a nethouse in which, treated plants directly planted in the soil.

### 1. Effectivity of Vaccine on Field Grown Hot Pepper.

This experiment would show the efficacy of vaccine in natural condition. Pepper plants were cultivated according to local farmer practices. Before transplanted in the field, 3 - 4 weeks old seedlings were vaccinated, and two weeks later they were planted in the field.

The experiments were executed in different location namely Segunung and Lembang for highland area, and Subang, Bekasi, Serpong, Kramat and Madura for lowland areas.

## B. Results And Discussion

### a. Distribution and Prevalence of Viral Diseases of Pepper

Samples were collected from four different islands of Indonesia. The symptoms of collected pepper samples varied in every location and time. In some locations symptoms were difficult to recognize due to symptoms caused by other damaging agents i.e insect pest and or fungal diseases. However, in the other areas symptoms were very distinctive, easy to recognize and to distinguish from other diseases (Photo 1). Mosaic, mottle, curling, defoliation, sinousity, necrosis and rugose were considered to be virus symptoms. Common symptoms of CMV on pepper is chlorosis, elongated of leaves and sinousity (Photo 2). Sometimes may show slight mosaic and puckering.

Photo 1 : Pepper field of farmer in East Java. Yellowing due to virus attack

Photo 2 : Common symptoms of CMV on hot pepper chlorosis, elongated of leaves and sinousity.

About thousand samples from different locations were tested. Viral symptoms were identified by indirect Elisa test as caused by CMV, PVY, TEV, CVMV and other viruses. Some samples did not react with any antiserum but some reacted with more than one antiserum. Sample with the same symptoms were sometimes diagnosed as having different viruses. These results might be due to different cultivars and different environmental conditions.

Pepper viruses were widely spread throughout the production areas. Viral infection were found in every location, but the intensity of its infection varied. However, most of the crop showed 100% infection of some viral diseases. Cucumber mosaic virus was the most common virus disease. The next was CVMV followed by PVY and other viruses.

## b. CMV Isolation and Determination of Its Strains

From tissue samples tested, CMV was isolated from mix infection of other viruses by using consecutive inoculation through *Nicotiana tabacum* Xanthi nc and *Cucurbita pepo*. Isolates of the virus obtained caused systemically chlorotic lesions on *C. pepo*, a specific or differential indicator plant of CMV.

These CMVs were grouped into 12 isolates. Based on the intensity, type of symptoms and incubation periods on their indicator plants they were determined into 6 types, namely CMV-1, CMV-2, CMV-4, CMV-5-8-10, CMV-11 and CMV-12 (Photo 3). They were 4 isolates out of 12 lost their infectivities.

Photo 3 : Symptoms of six isolates of CMV on *Nicotiana tabacum* Xanthi nc

c. Stimulation of Satellite RNA

Serial passage of six isolates on tabacco to develop satellite RNA were tested by PAGE analysis. Results showed that CARNA 5 band was detected after 5-6 serial transfers.

The development of, CARNA 5 on infected plants clearly changed the symptoms of CMV-2 and CMV-4, but not on other isolates. The initial symptoms on CMV-2 without CARNA 5 were mosaic and yellowing on tobacco. At every step of transfer the intensity of symptoms was gradually reduced. These symptoms were completely disappeared after CARNA 5 reach to detectable level (Photo 4 and 5). When CARNA 5 containing CMV 2 was reinoculated to healthy tobacco induced very mild symptoms or symptomless (Photo 6).

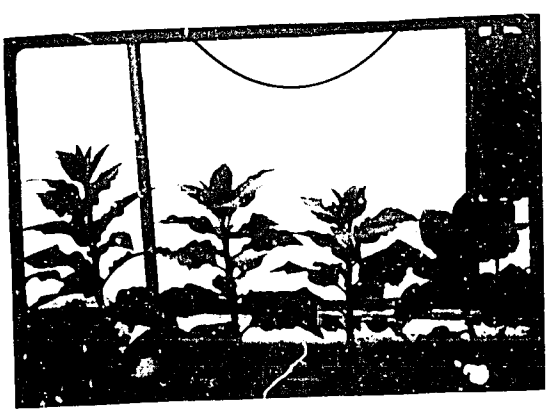


Photo 4. Stimulation of satellite CARNA 5 on serial passage to tabacco Xanthi : Symptom of original CMV is attenuated by rising satellite from left to right.

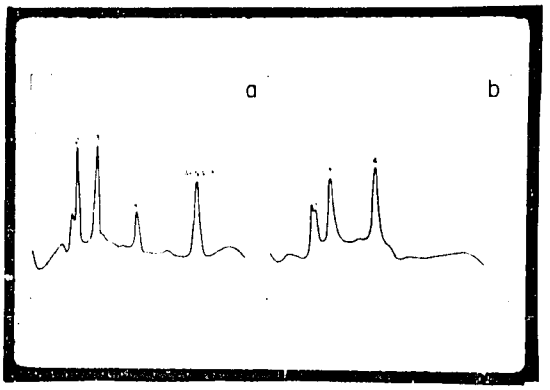
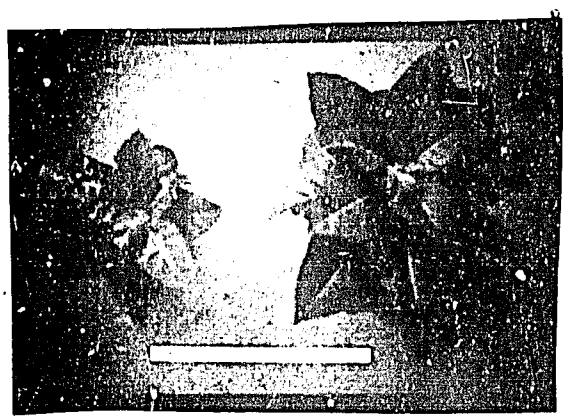



Photo 5 : Composition of viral RNAs of CMV.
a. From the plant with attenuated symptoms, showed a peak of RNAs.
b. From the plant with original CMV symptoms, no peak of RNA-5



a b

Photo 6 : Symptoms expression of CMV on tobacco Xanthi : a. without satellite
b. with satellite.

Tobacco plants infected by CMV-4 showed mild mosaic symptoms after 5-6 passages, when CARNA 5 was detectable the symptoms became milder. Since CMV-2 and CMV-4 appeared to be the best helpers for CARNA 5 multiplication, so the further studies have been focused on this two isolates. Later on due to the better response of CMV 2 vaccine, this strain becomes a highest priority.

## d. Virus Purification

CMV-2 and CMV-4 strains were propagated on several host plants and harvested at 10 to 14 days after inoculation. Each virus inoculum was purified with Lot method. The results indicated that the highest yield of purified CMV-2 was found from tobacco (table 1), while of CMV-2 + CARNA 5 from pepper and tobacco which harvested at 11 and 14 days after inoculation respectivelly. The yield of CMV-2 and CMV-2 + CARNA 5 from tomato was low. Tomato appeared to be not a good host for CMV-2 multiplication. However, the yield of CMV-4 + CARNA 5 from tomato plants was higher than with CMV-2, with or without CARNA 5.

Table 1 : Yields of Virus and Vaccine Purification

| Virus/ Vaccine | Propagative plant | Harvest time (days) | Virus yield mg / kg | Place |
|---|---|---|---|---|
| CMV 2 | Tomato (1x) | 12 | 13.78 | USDA |
| | Tobacco (1x) | 14 | 29.20 | USDA |
| | Tomato (1x) | 30 | 0 | LEHRI |
| | Tobacco (7) | 14-16 | 5.0-140.0 | LEHRI |
| CMV2+C5 | Tomato (2x) | 12 | 7.6-10.0 | USDA |
| | Pepper (1x) | 11 | 464.6 | USDA |
| | Squash (1x) | 8 | 16.34 | USDA |
| | Tomato (1x) | 13 | 0 | LEHRI |
| | Tobacco (3x) | 14 | 80-120 | LEHRI |
| CMV4+C5 | Tomato (1x) | 12 | 57.96 | USDA |

( ) = Number of purification was done

## e. Viral RNA extraction

The yield of RNA which was extracted from purified CMV of pepper was lower than purified CMV of tomato. The both yields were very low, concentrations were only 1.7 and 6.2 percent from the initial weight of virus. This activity was done in USDA.

## f. Fractionation of CMV RNAs and CARNA 5

CMV-2 + CARNA 5 from pepper plants was used in this study. Genomic RNA 1,2,3, coat protein massenger RNA 4 and CARNA 5 were recovered from sucrose gradients by a modification of Lot et al.(1974). Up to 308 ug of RNA were loaded on the sucrose gradient, roughly 25, 35, and 40% would be respectively CARNA 5, RNA-4 and RNA 1,2 and 3. This activity was done in USDA.

## g. Introduction of S-CARNA 5 into Indonesian CMV isolates

Result indicated that tomato plants infected with CMV-4 or CMV-12 showed systemic symptoms at one week after inoculation. CARNA 5 was detected at 2 weeks after inoculation. At 3 weeks after inoculation, double stranded CARNA 5 was detected. Based on this result both isolates can support S-CARNA 5 replication.

Symptoms of CMV-4 infected tomato plants with or without CARNA 5 were mild mottle. However, there were two out of ten plants showed systemic necrotic symptoms. The necrotic reaction of those plants appeared to be caused by mutant or micture of necrogenic CARNA5 with non-necrogenic S-CARNA 5. Symptoms of CMV-12 infected tomato plants without CARNA 5 were mosaic and fern leaf. Some necrotic areas developed on the leaves of the plants infected with CMV-12 with CARNA 5.

The occurence of S-CARNA 5 affected the symptoms caused by CMV-4 or CMV-12. CARNA 5 tends to attenuate the symptoms on plants infected by CMV-4. Most of the plants infected by CMV-4 showed mild to moderate symptoms. After introducing CARNA 5 to this isolate, the symptoms of infected plants became milder. In CMV-12, CARNA 5 intensified the yellowing symptom on tobacco and pepper California Wonder. The necrotic symptoms, however, appeared to be eliminated, and stunting was reduced by the presence of CARNA 5 on pepper infected plants. The symptom of CMV-12 on squash was also attenuated by the presence of CARNA 5.

## h. Evaluation of A Candidate Vaccine in Glasshouse

### 1. Evaluation of the vaccine for CMV disease protection on pepper

The main objective of this experiment was to determine the shortest period of the vaccine to be ready to protect plants from challenged inoculation.

This experiment yielded that Bell pepper inoculated with CMV-2 alone showed severe mosaic and necrotic symptoms, some plants died. However, the plants which were vaccinated with CMV-2 + CARNA 5 and then superinfected by CMV-2 in two weeks after vaccination (Photo 7) and four weeks after vaccination (Photo 8) showed very mild or even no symptoms at all. Disease Index was not more than 1. It was difficult to judge the effect of challenged inoculation at one week after vaccination. The TNA analysis exhibited that CARNA 5 could be detected in large amount at this stage. This means protection began at least one week after vaccination. No tendency of break down protection was observed until 4 weeks after vaccination.

Photo 7 : Symptoms expression of pepper plant inoculated with CMV2 at 2 weeks after vaccination.
Left : without vaccine (VoC2)
Right: with vaccine (V1C2)

Photo 8 : Symptoms expression of pepper plant inoculated with CMV2 at 4 weeks after vaccination
Left : with vaccination V1C4
Right: without vaccination VoC4

## 2. Satellite protection for controlling CMV on bell and hot pepper

Two inoculation of vaccine (CMV-2 + CARNA 5) namely purified one (20 ug/ml in 0.03 M $Na_2HPO_4$) and crude sap tissue (1 part of infected tissues in 9 part of 0.03 M $Na_2HPO_4$) were applied to Bell and Hot pepper at 14 days after germination. Two weeks later the infected seedlings were challenged

Results indicated that almost all treated plants (each treatment consisted of 50 plants) using purified vaccine looked healthy (Photo 9). One out of 50 treated plants of Bell

Photo 9 : Almost no differences between unvaccinated plant (VoCo, left) and vaccinated plant (V1Co, right)

pepper showed stem necrosis. From the infectivity test of inoculum on Zucchini squash indicated positive reaction were occured for both vaccine and challenge inoculation.

The protection experiment with crude sap showed that the vaccine can protect peppers from severe strain infection. However, some of vacccinated plants exhibited symptoms. The possible reason of this fact is the inhibition of satellite multiplication at lower temperatures.

### 3. Reaction of selected plant species to the candidate vaccine

The objective of this study is to determine the possibly adverse influence of the vaccine on various commercial crops. Inoculum of the vaccine used was sap from infected tobacco tissues homogenized in 0.03 M phospate buffer (1/10 w/v).

Results (table 2) showed that symptoms of CMV2 + CARNA5 on most of cucurbit plants were more severe that symptoms caused by CMV2 only. Local symptoms on *Vigna radiata* and *Chenopodium quinoa* were also intensified by the presence of CARNA 5. This finding is not common for most CMV + CARNA 5 reported from other countries. In China (Tien Po et. al., 1987) the vaccine could reduce the symptoms caused by CMV on various plant species from different families. In our case perhaps low ratio between satellite and CMV-2 in the inoculum and low temperature in the screenhouse during the experiment are the possible reason. More plant species should be tested. A purified or partial purified vaccine should be used for further tests.

Table 2. Reactions of Selected Crop Species to Inoculation with CMV-2 Without and With Carna5

| Plant species | Symptom | |
|---|---|---|
| | CMV-2 | CMV-2 + CARNA 5 |
| - *Vigna radiata* | LL | LL + |
| - *Cucumis sativus* | | |
| (Lokal Cianjur) | Cl | M + |
| ( Armenian ) | - | Cs, M |
| - *C. melo* Cantalope | Cl | Cs, M |
| - *Cucumis spp* | - | Cs, M |
| - *Cucurbita pepo* | | |
| (Zucchini squash) | Cs | Cs, M + |
| - *Cucurbita maxima* | Cs | Cl, M |
| - *Nicotiana tabacum* | | |
| (Havana) | YM | Cl |
| - *Chenopodium quinoa* | LL | LL + |

LL = local lesion, M = mosaic, - = no symptom, YM = Yellow mosaic, Cs = chlorotic spot, Cl = chlorosis, + = Intesified. Both inocula could not induce symptoms on : *Phaseolus vulgaris, Vigna sesquipedalis, Apium graviolens, Daucus carota Cucurbita moschata, Momardica charantea, Loofa cylindrica, Citrulus lanatus, Lycopersicon esculentum, Solanum tuberosum, S. melongena, Zea mays* (Sweet corn), *Brassica oleracea, B. chinensis, Chrysanthemum spp, Allium fistulosum.*

## 4. Effects of CMV vaccine to PVY and Indonesian CVMV

This experiment showed the possible synergism between the vaccine with other pepper viruses such as CVMV or PVY

Table 3 : Effects of CMV Vaccine to The Infection of PVY and Indonesian CVMV on Pepper, Segunung 1990

| Treatment | Disease index *) | Fruit yield g/plant | Yield increase (%) |
|---|---|---|---|
| Vaccine+0 | 0.33 | 965.5 | + 11.6 |
| Vaccine+CVMV | 1.92 | 479.8 | - 44.5 |
| Vaccine+PVY | 0.13 | 899.0 | + 3.9 |
| 0 + CVMV | 1.75 | 635.5 | - 26.5 |
| 0 + PVY | 0.17 | 501.0 | - 42.1 |
| 0 (Control) | 0 | 865.0 | |

*) Disease index : 0 - 5
0 = no symptom, 1 = chlorosis, 2 = mild mosaic
3 = fifty percent of plant showed mosaic
4 = mosaic, leaf malformation and stunted
5 = severe symptom : mosaic, heavy necrosis and died

(Table 3). Infection of CVMV alone or together with the vaccine induced the highest disease indice, but the disease index was sligthly lower if only CVMV alone. Transitory symptoms appeared on some vaccine treated plants. On the other hand the vaccine exhibited antagonistic reaction against PVY, and had considerable protective capability to this virus infection.

## i. Field Tests of CMV Vaccine

These experiments would show the efficacy of vaccine in natural condition. Pepper plants were cultivated according to local farmer's cultural practices. Before transplanted in the field, 3-4 weeks old seedlings were vaccinated, and two weeks later they were planted in the soil.

### 1. Control of CMV disease on pepper cv. Cipanas using satellite mediated cross protection under controlled field

This experiment was implemented as micro plots under a screen tunnel. Four treatments were applied namely vaccine of CMV2 + C5, vaccinated then challenged with common severe CMV, inoculated with common severe CMV and control (no vaccine and no challenger). Results summarized in Table 4, indicated that CMV vaccine could protect pepper plants from superinfection of the severe strain, even from other natural virus infection (compared with control). Pod production of vaccinated plants was the highest, while pepper plant infected with severe strain showed the highest disease intensity and the lowest height and the lowest pod production.

Table 4 : Influence of CMV Vaccine on Pepper cv. Cipanas under Controlled Field Condition

| Treatment | Disease intensity (%) | Plant height (cm) | Increase of height (%) | Fruit yield g/plant | Increase of yield (%) |
|---|---|---|---|---|---|
| Vaccine | 0.0 a *) | 100.2 b | + 11.3 | 239.4 b | +22.8 |
| Vac+sev.CMV | 5.0 a | 92.6 b | + 1.8 | 195.9 b | + 0.5 |
| Severe CMV | 75.3 b | 67.9 a | - 25.3 | 99.2 a | -49.1 |
| Control | 8.7 a | 90.9 b | 0 | 194.9 b | 0 |

*) The same letter indicated not significant different at 5% DMRT

### 2. Effect of CMV vaccines on field grown pepper

Field experiments were done in different locations using some CMV-vacines and some cultivars. Results were summarized in table 5. Expression of the plants varied among locations

Table 5 : Influence of CMV Vaccine on Disease Intensity and Field Production of Pepper (1990/1991)

| Location and pepper cultivar | Treatment | Disease intensity (%) | Fruit yield kg/10 plants | Yield Increase (%) |
|---|---|---|---|---|
| a. Lembang | | | | |
| cv. Tit Super | Control | 2.30 | 2.337 | 0 |
| | CMV2 + C5 | 4.38 | 2.697 | + 15.4* |
| | CMV4 + C5 | 4.60 | 2.447 | + 4.7 |
| | CMVS + C5 | 3.03 | 2.562 | + 9.6 |
| b. Subang | | | | |
| cv. Barito | Control | 34.7 | 0.937 | 0 |
| | CMV2 + C5 | 33.9 | 1.524 | + 62.63* |
| | CMV4 + C5 | 31.4 | 1.067 | + 13.84 |
| | CMVS + C5 | 30.5 | 1.220 | + 30.18 |
| c. Tegal | | | | |
| cv. Jatilaba | Control | 24.64 | 0.090 | 0 |
| | CMV2 + C5 | 30.82 | 0.096 | + 7.01 |
| | CMV4 + C5 | 27.77 | 0.117 | + 30.08 |
| | CMVS + C5 | 27.96 | 0.095 | + 5.98 |
| d. Madura | | | | |
| cv. Jatilaba | Control | 53.41 | 1.113 | 0 |
| | CMV2 + C5 | 53.52 | 1.289 | + 15.77* |
| | CMV4 + C5 | 54.79 | 1.200 | + 7.84 |
| | CMVS + C5 | 52.84 | 1.279 | + 14.90 |
| e. Bekasi | | | | |
| cv. Cipanas | Control | 28.93 | 1.305 | 0 |
| | CMV2 + C5 | 16.00 | 1.847 | + 41.5* |
| | CMV2+C5,chal | 17.75 | 1.472 | + 12.7 |
| | Challenger | 19.09 | 1.317 | + 0.9 |
| f. Segunung | | | | |
| cv. L 1603 | Control | 19.82 | 2.414 | 0 |
| | CMV2 + C5 | 12.41 | 3.146 | + 23.2* |
| cv. L 2175 | Control | 12.18 | 1.998 | 0 |
| | CMV2 + C5 | 6.89 | 1.628 | - 18.5 |
| cv. L 1559 | Control | 20.53 | 1.880 | 0 |
| | CMV2 + C5 | 11.83 | 2.324 | + 23.6* |
| cv. L 1689 | Control | 37.48 | 0.716 | 0 |
| | CMV2 + C5 | 20.28 | 0.750 | + 4.7* |
| cv. L 2320 | Control | 24.52 | 1.486 | 0 |
| | CMV2 + C5 | 11.21 | 2.818 | + 89.6* |
| cv. L 1492 | Control | 28.46 | 2.126 | 0 |
| | CMV2 + C5 | 22.02 | 1.516 | - 28.6 |
| cv. Jatilaba | Control | 11.00 | 2.344 | 0 |
| | CMV2 + C5 | 8.25 | 2.788 | + 18.9* |
| cv. Cipanas | Control | 20.98 | 2.064 | 0 |
| | CMV2 + C5 | 12.76 | 2.702 | + 30.9* |
| cv. Hot Beauty | Control | 28.25 | 2.708 | 0 |
| | CMV2 + C5 | 21.99 | 2.654 | - 1.9 |
| cv. Keriting genjah | Control | 22.53 | 2.156 | 0 |
| | CMV2 + C5 | 21.80 | 2.292 | + 6.3* |

* CMV2 + C5 showed better protection

and pepper cultivar. It may be due to different conditions and other cinterferences (other diseases and pests) besides CMV. In Subang, Tegal and Bekasi experiment plots were highly attacked by mite and thrips. However, comparing to control, vaccination always gave better fruit yields, except in Segunung for cultivar LV 2175, LV 1492 and Hot Beauty. In general, vaccine of CMV 2 + CARNA 5 was better than two others (CMV4 + CARNA 5 and CMVS + CARNA 5).

On-farm research should be continously carried out to support the improvement of the method in large scales. The variability of pepper cultivars grown by farmers and the differences of micro climatic conditions in pepper- growing areas are the main consideration to establish succesful application of the vaccination. Besides that, the next future work has to be concentrated in how to develop methods of rapid propagation of the vaccine to be used as a ready-product for farmers.

## IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

Peppers are grown in large range of climate from lowland to highland in Indonesia. In 1987 pepper production areas recorded was up to 264,000 hectares (Dir. Bina Prod., 1987), and the average yield around 2 ton/Ha. It was very low compared to its potential yield 8-15 ton/Ha.

Until now virus diseases on pepper is the second important problem after fruit-rot antracnose. Cucumber mosaic virus has been one of the major constraints in pepper production in Asean countries. The application of biological control by using protective CARNA 5 against CMV on peppers would be very useful in the field in which this virus is prevalent. It may be useful for other pepper virus like PVY. This finding is not only beneficial for Indonesia but also for other countries which have similar problem.

At this moment the field tests of CMV-vaccine has not been completed due to interference by other factors, so the application trials of the vaccine should be continously through integrated plant protection with other approach to establish a sustainable of vaccination. A consistent result of large scale trials will warrant to get the proper application of vaccine.

The researhers involved in this project and LEHRI as the primary Indonesian Goverment Institute for horticultural crops have taken benefits of the technology transfer during the project period in terms of the improvement of knowledge and supporting equipment for research purposes. This project has generated a conceptual consideration for developing biological control of other plant virus diseases. The virologists of the institute have obtained additional capacity of research methodologies and technologies of molecular plant viruses. The project provided a first opening towards the newly emerging biotechnologies. In the longer term this could also mean that Indonesian scientists within

their own agricultural research organizations would have an incentive to cooperate in this more basic efforts with the U.S. institutions.

PROJECT ACTIVITIES/OUTPUTS

A. Meeting/Training

a. Overseases Training and Seminar

| Date and place | Title | No. of LEHRI's participant |
|---|---|---|
| Jun.88 - Jul.89 USDA Maryland, US | Molecular plant virus | 1 |
| May 31-Jun.27, 89 AVRDC, Taiwan | Workshop of pepper viruses | 2 |
| June 5-July.17,89 USDA Maryland, US | Molecular plant virus and TNA analysis | 1 |
| Oct 1990 ( 3 wks ) AVRDC, Taiwan | Training of plant viruses | 2 |
| March 15-16, 1991 Kualalumpur | AVNET ; Pepper viruses | 1 |

b. Local Training / Seminar of Using Viral Satellite for Controlling CMV

1. November 24-26, 1987 in Surabaya : Congress of Indonesian Phytopathological Society IX. Speakers : Duriat and Sulyo. Participants attended 40 persons.

2. Februari 10-24, 1988 in Lembang, Bogor, Yogya and Malang. Seminar : Viral Satellite of CARNA 5. Speakers : Kaper, Duriat, Muharam. Participants attended 30-40 persons.

3. December 7-9, 1989 in Denpasar, Bali. Congress Indonesian Phytopathological Society X. Speaker : Sulyo, Duriat, Sutarya. Participants attended 40 person.

4. March 8-9, 1990 in Segunung. Training : TNA Analysis. Instructor : Kaper and Sulyo. Participants attended 3 person from LEHRI.

5. March 10, 1990 in LEHRI, Lembang. Seminar : Progress Report of CMV Research, and Successes of Recent Field Test of CARNA 5 in Italy. Speaker : Duriat and Kaper. Participant attended 20 person from LEHRI and ATA-395 Project.

6. March 13, 1990 in USAID Jakarta. Seminar : Progress Report of CMV Research, and Satellite-mediated Symptom Modulation Speaker : Kaper and Duriat. Participants attended 7 person.

7. June 11-December 11, 1990 in AARD, Bogor. Training : Plant Biotechnology. Instructor : Duriat, Sulyo and Sutarya. Participants attended 12 person from other Research Institutes.

8. September 8-10, 1990 in Balittas, Malang. Seminar : Control of CMV using CARNA 5. Speaker : Duriat. Participants attended 30 person from Tobacco Research Institutes.

9. October 1, 1990 - February 28, 1991 in LEHRI, Lembang. Training Purification of Virus and Vaccine CMV. Instructor : Duriat and Sutarya. Participant attended 1 person from Tobacco Research Institutes (Balittas) in Malang.

10. May 16-30, 1991 : in Bogor : Regional Training Course on Screening against Disease and use of Biotechnology for Detection of Pathogens. Speaker : Duriat. Title of presentation: The use of Biotechnology of Crop Disease management The case of CARNA 5. Participants attended 25 persons from Asean Countries.

11. May 21-24. 1991 in Bogor : Workshop on Agricultural biotechnology. Speaker : Duriat. New approach of plant Biotechnology for controlling Cucumber Mosaic Virus on Pepper. Participants attended about 110 persons from various institution in Indonesia and other countries.

**c. Technical Assistance (1.60 PM)**

1. February 3-26, 1988. Technical Assistance : Dr.J.M. Kaper. Institution : USDA. Expertise Biochemist. (0.80 PM).

2. February 22 - March 15, 1990. Technical Assistance : Dr.J.M. Kaper. Institution : USDA. Expertise Biochemist. (0.80 PM)

**B. Publications**

1. Duriat, A.S., Y. Sulyo, and Agus Muharam. 1988. Distribution and prevalence of CMV on field-grown peppers and tomatoes. Internal Report LEHRI.

2. Duriat, A.S., Y. Sulyo, Agus Muharam and R. Sutarya. 1990. Biological control of cucumber mosaic virus (CMV), an important disease-causing agent on horticultural crops in Indonesia. Presented at a seminar in LEHRI, Lembang, 10 March 1990.

3. Duriat, A.S. 1989. Status of pepper virus diseases in Indonesia. Presented at The germplasm Workshop in AVRDC San Hua, Taiwan.

4. Sulyo, Y. 1989. Control of CMV on Tomato through Cross Protection (Pengendalian CMV pada tomat dengan proteksi silang), Proc. 10 th Congress IPS, Denpasar, Bali.

5. Sutarya, R. and A.S. Duriat. 1989. Preliminary study : Efficacy of Vaccine CMV-2 on Tomato Variety Berlian (Uji Pendahuluan vaksin CMV 2 pada tomat Berlian). Internal Report, LEHRI.

6. Duriat, A.S. and R. Sutarya. 1989. The Influence of CMV Vaccine on Host Pepper Variety Keriting and Barito (Pengaruh vaksin dan isolat CMV terhadap pertumbuhan dan produksi cabe varietas keriting dan Barito). Bull. Penel. Hort,LEHRI (in press).

7. Purbadi, supervised by Y. Sulyo. 1990. Effect of The CMV Vaccine Inoculation on The Disease Index of CVMV and PVY in Peppers (Pengaruh vaksin terhadap infeksi CVMV dan PVY pada Cabai). Thesis, Sekolah Tinggi Pertanian, Bandung, West Java.

8. Duriat, A.S. and R. Sutarya. and N. Gunaeni. 1990. Respons of 33 selected accession of hot peppers to the inoculation of CMV, PVY and mix of them. Internal Report, LEHRI.

9. Duriat, A.S., Y. Sulyo and R. Sutarya 1991. Virus Diseases Research of Pepper. Paper presented in mid-term Workshop of Asean Vegetable Net-Work. March 15-16, 1991 in Cameron Highlands Malaysia.

10. Sulyo, Y. 1991. Satellite RNA for CMV Diseases Protection. Sending to IMPACT, Scientific magazine in AVRDC, Taiwan.

11. Sulyo, Y and Suyatno. 1990. Effect of The Candidate Vaccine on Selected Crop Species. Internal Report.

12. Sutarya, R., A.S. Duriat and E. Korlina. 1991. Vaccination of Weak Strain of CMV on Pepper plant cv. Jatilaba in Kramat, Tegal (Vaksinasi dengan menggunakan strain lemah CMV pada tanaman cabai cv. Tit Super di Kramat, Tegal). Manuscript for. Bull. Penel. Hort. 1991.

13. Sutarya , R., A.S. Duriat and N. Gunaeni. 1991. Influence of Vaccination of Three Weak Strain of CMV on Pepper cv. Barito in Subang (Pengaruh vaksinasi dengan menggunakan tiga macam strain CMV lemah pada tanaman cabai varietas jatilaba di Subang). Manuscript for. Bull.Hort. 1991.

14. Sulyo, Y., A. Muharam, Suyatno. 1991. Control of CMV Disease on Pepper by Using Satellite Mediated Cross Protection. Manuscript for. Bul. Penel. Hort. 1991.

15. Sulyo, Y and Suyatno. 1991. Effectivity of Three Different vaccines on CMV disease Protection on Field Grown Peppers Internal report.

16. Duriat, A.S., R. Sutarya and E. Korlina. 1991. Influence of CMV Vaccine on Pepper in Margahayu Lembang (Pengaruh penggunaan vaksin CMV pada cabai di Margahayu, Lembang). Manuscript for Bul. Penel. Hor. 1991.

17. Duriat, A.S., Y. Sulyo., R. Sutarya and A.A. Asandhi. 1991. New Approach of Plant Biotecnology for Controlling Cucumber Mosaic Virus on Pepper. Paper presented in Workshop on Agricultural Biotechnology, CRIFC Bogor May 21-24, 1991. Indonesia

18. Sulyo. Y., A. Muharam., R.Sutarya and A.S. Duriat. 1991. Effect of CMV vaccine on 10 hot pepper varieties. Manuscript for the Congres IPS XI in Ujung Pandang. Indonesia

## PROJECT PRODUCTIVITY

LEHRI requested USAID to extend the project assistance completion date (PACD) from December 31, 1990 to June 30, 1991 in order to enable LEHRI to complete the planned project objectives which were, at that time, behind schedule due to the delays in the procurement of majcr laboratory equipment and technical problem of the main unit of ultra centrifuge. Unfortunatelly, USAID did not approve this request. This project had previously been extended twice. Since September 1990, most of the laboratory activities stopped and were succeded by field trials to evaluate candidate vaccines.

Not all the proposed objectives of the project activities during the life of the project were achieved successfully such as RNAs extraction and separation of CARNA 5. However most of the activities were accomplished.

Although it is realized that CMV is the important disease-causing agent of peppers, results of the field trials clearly showed that natural virus infection was not caused only by CMVbut also by other viruses in addition to sucking insect and other diseases. The vaccine was able to protect the plants against CMV and to inhibit PVY infection, but was not effective against other pepper-infecting viruses. The field trial in Madura exhibited a phenomenon that inter-cropping of vaccinated peppers with other crops such as corn decreased the infection of other aphid-transmited viruses (Photo 10).

Studying of CMV-RNAs and integrated efforts of the vaccination with the cultural techniques and control measures of aphid vectors is interesting to be further studied.

Photo 10 : Corn incombination with CMV vaccine may reduce the infestation of other sucking insects.

## FUTURE WORK

LEHRI continues research activities related to the production of the vaccine and the application of the vaccination at the farmer level. To support a smoot flow of technology transfer from the institute to pepper growers in terms of the vaccination concept, the involvement of the private sectors and other agricultural institutions such as Education and Extension Service is essential.

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Lembang June 2, 1991