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Selection of Mild Virus Strains for Controlling Yellow Mosaic Disease of Yard Long Bean

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ABSTRACT

Mild strains of cowpea aphid-borne mosaic virus (CAMV) were selected by field selection mutagenesis. An attempt to select mild strain from natural field was failed. Mutagenesis by heat or cold treatment gave one and five mild virus strains, respectively. Nitrous acid treatment induced thirteen strains of mild virus. Four of these nineteen mutants showed different level of cross protectivity when tested under greenhouse condition. The highest cross protection effectiveness was 75% with strain 37C-30D obtained from heat treatment mutation.

INTRODUCTION

Yellow mosaic disease of yard long bean caused by cowpea aphid-borne mosaic virus (CAMV) is considered to be one of serious disease because it decreased about 20% of production yield as measured by dry pod weight, number of pod per plant and seed weight (Jarupat and Sutabutra, 1978, Fahrungsang, 1984). The control measure used nowaday such as insecticide spraying to eliminate aphid vector was not so effective and had some disadvantage to farmers. Cross protection using mild virus strain has been considered to be a valuable approach to control virus disease in the field, as were reported for tomato mosaic disease in the Netherlands, the United Kingdom and Japan (Fletcher, 1975; Oshima, 1975;Nagai, 1988), citrus tristeza virus of sweet orange in Brazil (Costa and Muller, 1980), and papaya ringspot virus of papaya in Taiwan (Yeh *et al.*, 1986).

This study aimed to select mild strain of CAMV that can be used as an immune to protect yellow mosaic disease in yard long bean plant. Natural selection and mutagenesis were performed to produced mild virus strain. The effectiveness for cross protection of selected mild strains were tested under greenhouse condition.

MATERIAL AND METHODS

Virus purification : CAMV was isolated from yard long bean plants showing yellow mosaic symptom (Fig. 1) by single lesion isolation technique (Kado and Agrawal, 1972). The virus was propagated in yard long bean plants and purified by the method of Iwai and Wakimoto (1985) with some modifications. Yield of purified virus was about 10 mg per kg leaf tissues.

Production of antibody against CAMV : Antibody was produced in a rabbit by four intramuscular injections of purified virus at two weeks intervals. Antisera were collected every week after last injection. The titer of antisera obtained were determined by microprecipitin test. The high titer antiserum was further purified to get gamma-globulin for ELISA test in succeeding experiments.

Search for mild strains of CAMV from natural field plants : Symptomless yard long bean plants found within CAMV-heavily infected plots in Nakhon Pathom and Rachburi Provinces were col-

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lected. Screening for the presence of CAMV in plant samples was performed by serological reaction and electron microscopy. CAMV strains were isolated by single lesion isolation on *Chenopodium amaranticolor*. Mild virus strains were selected according to mild symptom development on yard long bean plant after individual lesions transfer.

Mutagenesis of severe strains of CAMV : Yard long bean plants inoculated with CAMV were exposed to heat treatment at 37°C or cold treatment at 15°C in growth chambers for about a month. Mutants were isolated by the same technique mentioned above. Another mutagenic treatment was done by using nitrous acid to induce mild virus strains (Siegel, 1965). The procedure used was the same as described by Chaichuchote (1987). Purified CAMV, concentration about 0.5 mg/ml, was pipetted to 500 µl and put into 1.5 ml microtubes. Freshly mixed solution of 4 M sodium nitrite and acetate buffer, pH 4.0, each of 250 µl, was added to each tube and mixed well with virus suspension. The reaction tubes were individually kept at room temperature for 10, 20, 30, 50, 60 and 120 min. At each time intervals, the reaction was added with 0.1 M phosphate buffer, pH 7.0 to make final volume of 2.5 ml. Treated virus was then inoculated onto *Chenopodium amaranticolor*. Single lesions developed were separately inoculated on yard long bean plants for symptom observation. Multiplication of CAMV in plant was determined by ELISA (Clark and Adams, 1975).

Cross protection test : Selected mild virus strains were tested for efficiency to cross protect CAMV severe strain under greenhouse condition. Crude sap from plant infected with mild strain of CAMV was firstly inoculated onto young seedling of yead long bean. CAMV serve strain was challenge inculated one month later. Symptom observation was done for two months and efficacy for cross protection was evaluated base on symptom appearance of plants.

RESULTS AND DISCUSSION

Virus purification and antiserum production : Yield of purified virus was about 10 mg per kg leaf tissues. The highest titer of antiserum obtained was 1/256 by microprecipitin test. Gamma-globulin was used at concentration of 1 µg/ml in ELISA test with sap dilution of 1/500. Enzyme-substrate reaction was terminated after 1 h incubation at room temperature.

Search for mild virus strains from natural field plants : Some of selected strains obtained from single lesions showed mild mosaic symptom on yard long bean plants. However, the second passage of these strains to new plants resulted in severe mosaic symptoms. Efforts to select mild strain from field plants were unsuccessful. Deema *et al.* (1978) also reported on attempts to select mild strains from natural field, but their strains were not mild enough to be used.

Mutants from mutagenic treatments : Five mild virus strains were selected from 107 lesions obtained after cold treatment of yard long bean plants. The mild strains were named 15C-7D1, 15C-7D4, 15C-3OD21, 15C-30D55, and 15C-30D65. Only one strain, 37C-30D, was selected from heat treated plant. Two mild virus strains, 10M-2, 40M-2, from thirteen nitrous acid mutants showed very faint mosaic symptom and were selected for cross protection test. These eight mild mutants were maintained in yard long bean plants, confirmed for virus multiplication, and tested for cross protectivity with CAMV servere strain.

It is interesting that mild virus strains selected in this study caused very faint mosaic symptom on yard long bean plant, but ELISA test indicated high concentration of the virus in plant. This corresponds to the result reported by Yeh *et al.* (1986) that symptomless infection on papaya caused by mild strain of papaya ringspot virus was not due to low concentration or slow multiplication of virus as indicated by strong positive reaction with ELISA test.

Effectiveness of mild virus cross protection in greenhouse condition : Yard long bean plant inoculated with severe strain of CAMV alone showed typical yellow mosaic symptom two weeks post inoculation. On the contrary, yard long bean plant firstly inoculated with some mild virus strains developed only faint mosaic or mottle symptoms 30 days after challenge inoculation with severe CAMV strain. Result from preliminary test indicated only four from eight mild strains that had cross protection potential (Table 1). Subsequent experiment was then conducted using strains 15C-30D21, 37C-30D, 10M-2, and 40M-2. (Fig. 2) The percentages of cross protection were determined 30 days after challenge inoculation. The result was summarized in Table 2. The strain 37C-30D offerred about 75% cross protected plants under

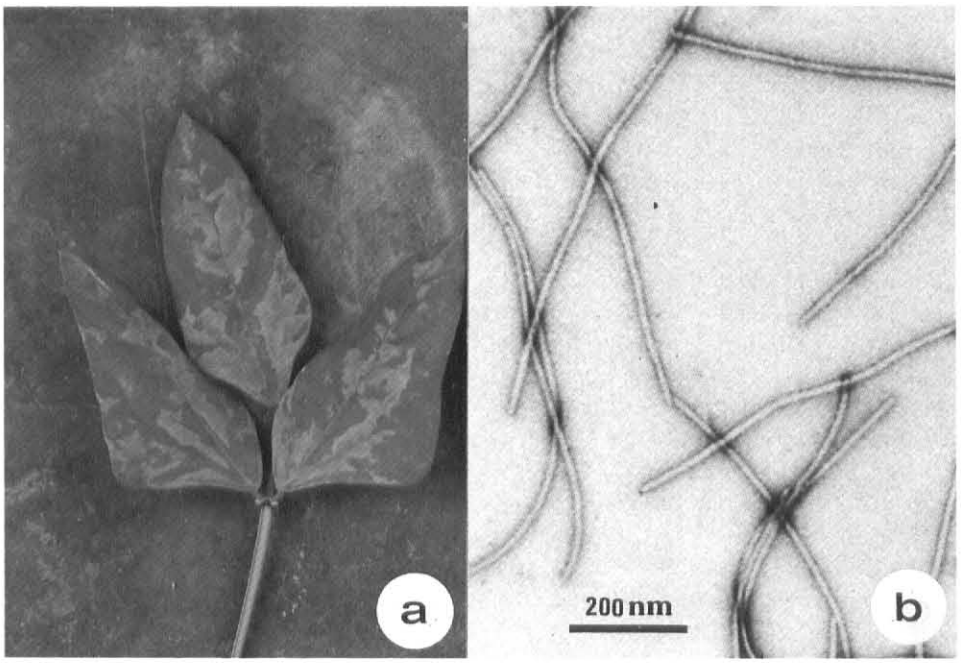
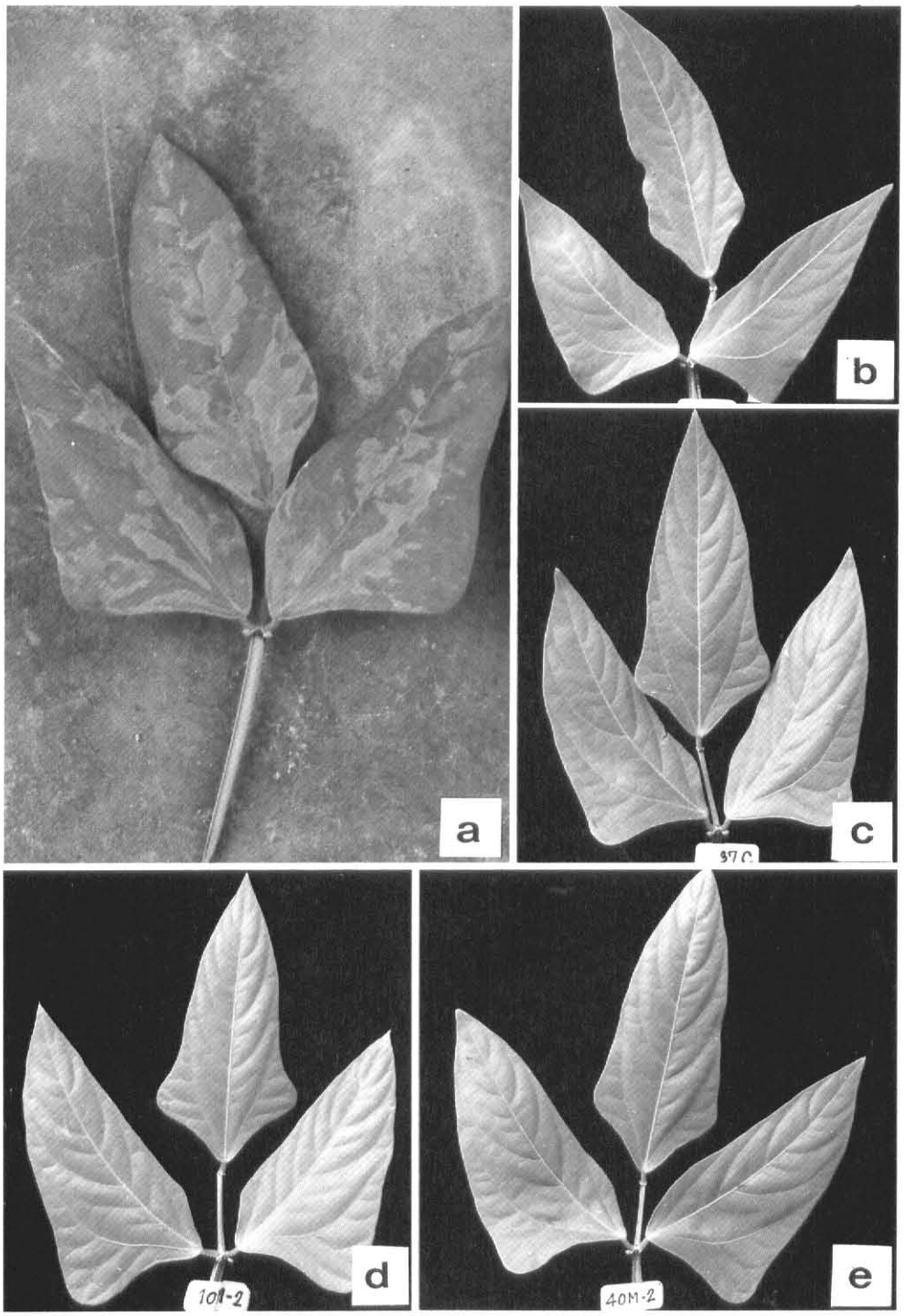


Figure 1 Typical yellow mosaic symptom on CAMV infected yard long bean (a), and morphology of virus particles (b).

greenhouse condition (temperature 25°C, 14 h daytime). Leaves and plant height of double inoculated plants did not look different from healthy ones (Fig. 3). Plants remained protected 60 days after challenge inoculation. Some young leaves developed after this showed yellow mosaic symptom of typical CAMV infection.

The results suggested that mild virus strains used in the test had some interfering effect on multiplication of severe virus strain. Further experiments of efficacy of the strain 37C-30D to protect yard long bean from yellow mosaic disease in the field remain to be conducted. In addition, it is worth to continue selection for higher effective strain that can provide complete or higher percentage of cross protectivity against CAMV severe strain.

Figure 3 Symptomless yard long bean plants inoculated with mild strain 37C-30D before challenge inoculated with severe strain. (Photograph:1 month after challenge inoculation).



ure 2 Symptoms on yard long bean plants inoculated with severe (a), and mild virus strains 15C-30D21 (b), 37C-30D(c), 10M-2 (d), and 40M-2 (e).

Table 1 Preliminary cross protection test to select mild virus strains which had potential to protect CAMV severe strain.

No.	Name of mild strain	No. of protected plant per No. of inoculated plant
1[a]	15C-7D1	0/5
2	15C-7D4	0/5
3	15C-30D21	3/5
4	15C-30D55	0/5
5	15C-30D65	0/5
6	37C-30D	4/5
7	10M-2	3/5
8	40M-2	4/5

No. 1-5, mutants from cold treated plants
No. 6, mutants from heat treated plants
No. 7,8 mutants from nitrous acid treatment

Table 2 Efficiency of mild mutants to protect yard long bean plants from CAMV severe strain.

Mild strain	No.of protected plant per No.of inoculated plant	% cross protection
15C-30D21	8/20	40
37C-30D	15/20	75
10M-2	9/20	45
40M-2	10/20	50

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