Screening and Identification of Ridge Gourd [Luffa acutangula (L.) Roxb.] Genotypes against Cucumber mosaic virus (CMV) Tolerance

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ABSTRACT

An experiment was carried out on screening of ridge gourd genotypes against cucumber mosaic virus (CMV). This study was conducted during 2011 – 2012. Thirty five ridge gourd genotypes obtained upon mechanical (artificial) inoculation were evaluated for CMV disease severity by using DAS –ELISA method. Among the thirty five ridge gourd genotypes, 12 genotypes of IC 92685 (0.139), IC 385912 (0.140), IC 3922334 (0.152), Coimbatore local (0.141), UP variety local (var:100) (0.138), SG 030 (0.193), K 090 (0.183), IC 413592 (0.124), IC 373361 (0.126), IC 362481 (0.149), IC 413577 (0.120) and LA 1 (0.173) had lesser OD values than control cowpea sample (0.613) and were found to be negative for CMV and considered as phenotypically tolerance. These genotypes were completely symptomless during mechanical inoculation with leaves showing CMV symptoms.

Keywords: Ridge gourd, Germplasm, CMV virus, Tolerance, Susceptibility, DAS - ELISA.

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Introduction

Cucurbitaceous vegetables are more prone to virus diseases. More than 20 viruses, including Cucumber mosaic virus (CMV), Watermelon mosaic virus-2 (WMV2), Papaya ring spot virus-W (PRSV-W) (formerly WMV1), Squash mosaic virus (SqMV), Zucchini yellow mosaic virus (ZYMV) and Cucurbit aphid-borne yellows virus (CABYV) have been reported to cause diseases in major cucurbit growing areas of the world. It was reported by many earlier workers viz., Lovisolo (1980), Purcifull et al., (1984), Gu et al., (2002), Papayiannias et al., (2005), Ozaslan et al., (2006), Bashir et al., (2006) and Massumi et al., (2007). The Cucumovirus (CMV) and the Potyviruses (WMV-2, PRSV-W and ZYMV) are the most frequent viruses infecting melon crops, worldwide (Alonso et al., 2003).

Several viruses viz., Cucumber mosaic virus (CMV), Watermelon mosaic virus-1 (WMV-1)/Papaya rings spot virus-W (PRSV-W), Zucchini yellow mosaic virus (ZYMV),
Cucumber green mottle mosaics virus (CGMMV), Watermelon mosaic virus-1 (WMV-2) and Yellow vein mosaic virus of pumpkin (YVMV) infecting cucurbits have been reported from India, Nepal, Taiwan, Pakistan and Sri Lanka (Bhargava and Joshi 1960), Dahal et al., (1997), Wang and Yeh (1998), Cheema et al., (1999) Ali et al., (2004) and Ariyaratne et al., (2005).

Occurrence of cucumber mosaic virus (CMV) is distributed worldwide. In Turkey, field incidence of cucumber mosaic virus (CMV) and zucchini yellow mosaic virus (ZYMV) in cucurbits was reported as 36.8 per cent and 39.2 per cent respectively (Ozaslan et al., 2006). The virus causes the most severe disease in muskmelon, squash and snap melon at early stages of crop by producing blistering symptoms (Sharma et al., 2007). Sandhu and Kang (2007) reported that strains of cucumber mosaic virus (CMV) and watermelon mosaic virus-1 (WMV-1) caused mosaic syndrome in cucurbits in Punjab.

Therefore, to evaluate and catalogue sources of CMV resistant genotypes, thirty five ridge gourd genotypes were screened by mechanical inoculation. The level of resistance to CMV accumulation in ridge gourd leaf tissues were evaluated using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) method.

**Materials and Methods**

**Virus source and maintenance**

Leaves of ridge gourd showing symptoms of mosaic, blistering vein thickening and leaf distortion were collected from ridge gourd germplasm evaluation block in the college orchard, Tamil Nadu Agricultural University (TNAU), Coimbatore. From this evaluation block, the major symptoms related to CMV were observed. For confirmation of physiological symptoms, these infected leaves were used as the source of inoculum for testing the virus by different CMV host plants of leguminaceae family viz., cow pea, lab lab and cluster bean (Plate 1) were used by mechanical inoculation method under controlled condition.

**Mechanical inoculation**

Ridge gourd seeds were sown in the pot mixture containing mixture of red soil: sand: farm yard manure at 1:1:1 w/w/w under insect proof cages in the glass house. One gram of infected leaf was ground in a pre-chilled mortar and pestle using 1 ml of 0.1M chilled sodium phosphate buffer (pH 7.2) containing β-mercaptoethanol and 0.01 M EDTA. The sap was rub inoculated using the pestle at 3-leaf stage previously dusted with carborandum powder of 600 mesh. After five minutes the excess sap was washed off with distilled water and plants were observed for symptom expression.

After confirmation of CMV virus infection from the host plants of cow pea, lab lab and cluster bean, the sap inoculation was carried out from ridge gourd to 35 ridge gourd genotypes of the study and similarly from ridge gourd germplasm to cow pea (Plate 2) at cotyledon stage, *i.e.* before the first true leaf development. Inoculated seedlings began to show symptom expression after 13-15 days. The inoculated plants exhibited leaves with mosaic pattern and made the leaves crinkled and miss happened. Twenty five days of post inoculation period leaves of cow pea and ridge gourd leaves were subjected to DAS-ELISA.

After confirmation, the inoculated host plants were maintained and used as a source for CMV and mechanical inoculation to the thirty five ridge gourd genotypes as above mentioned procedure. Twenty five days post
inoculation, the thirty five ridge gourd genotypes leaves were subjected to DAS –ELISA in order to determine which genotype express resistance towards CMV (Plate 3 and 4).

**Serological assay (DAS-ELISA)**

DAS-ELISA (Double Antibody Sandwich - Enzyme linked immunosorbant assay) was performed for detection of the virus isolates following the manufacturer’s instruction (DSMZ GmbH, Braunschweig, Germany). Purified IgG was diluted in coating buffer (1:1000) and 200 μl was added to each well of a microtiter plate. The plates were incubated at 37°C for 3 hours and thereafter plates were washed with phosphate buffered saline-Tween (PBS-T), for 2 – 3 minutes with two to three washings. Plates were tapped upside down on tissue paper. Aliquots of 200 μl each of the test sample extracted in sample extraction buffer was added to wells in duplicate. Buffer without sample served as control and plates were incubated at 4°C overnight. The plates were washed as earlier and 200 μl of the anti-virus conjugate (1:500) was added to each well and incubated at 37°C for 2 hours. Later the plates were washed three times. Finally 200 μl of freshly prepared substrate (10 mg of p-nitro phenyl phosphate dissolved in 10 ml of freshly prepared substrate buffer) was added to each well and incubated in dark at room temperature for 45 minutes or as long as necessary to obtain clear reactions. Spectrometric measurement of absorbance was read at 405 nm (EL 800, BIO-TEK Instrument Inc., USA) and the reaction was later arrested by adding 50 μl of 3M NaOH.

**Results and Discussion**

Cucumber mosaic virus (CMV) is one of the most wide spread viruses in the world infected over 1200 species belonging to more than 100 families of dicotyledonous and monocotyledonous plants (Edwardson and Christie, 1991; Roossinck, 2002). Palukaitis and Garcia-Arenal (2003) reported that the virus was transmitted in the laboratory by mechanical inoculation with plant sap and naturally transmitted by more than 80 species of aphids in a non circulative manner. Viruses infecting cucurbits produce different types of symptoms. Since, some of them cause similar symptoms and it becomes difficult to differentiate such viruses based on symptom expression. CMV infected plants of cucumber, squash and pumpkin also exhibits leaf mosaic, leaf distortion, fruit mosaic, stunting and mottling (Bashir et al., 2006).

Upon mechanical inoculation of the seventeen cucumber inbred lines with CMV isolate, eleven cucumber genotypes showed mosaic symptoms at the cotyledon stage. Symptoms developed later on, included severe systemic mosaic and yellowing of young leaves and crinkling and blistering in the older ones (Khereba et al., 2009).

Plants infected early in the season were severely stunted and leaves were malformed, and fruit were unmarketable because of pronounced rugosity (roughness) on the fruit surface, as shown on the infected zucchini plant and muskmelon which had shown severely stunted growing tips (Medalin Chiuoaru et al., 2012). Most of the commercial cultivars of cucumber were susceptible to the virus and when infected would show mosaic, motting and distortion of leaves and fruits (Parichat et al., 2014).

The OD value for CMV infected samples of cowpea, Lab lab and cluster bean were recorded as 0.560, 0.741 and 0.452 respectively with CMV antiserum at 405 nm. Source of inoculated plant sample of cowpea and ridge gourd were recorded as 0.562 and 0.743 respectively with CMV antiserum at 405 nm.
Table 1 Screening of ridge gourd genotypes against CMV by DAS – ELISA O.D. values

| Genotypes                          | O.D. value at A_{405} nm | Results |
|------------------------------------|--------------------------|---------|
| IC 92618                           | 0.479                    | +ve     |
| IC 92660                           | 0.475                    | +ve     |
| IC 92685                           | 0.139                    | -ve     |
| IC 105554                          | 0.517                    | +ve     |
| IC 105579                          | 0.552                    | +ve     |
| IC 110892                          | 0.603                    | +ve     |
| IC 196589                          | 0.549                    | +ve     |
| IC 339239                          | 0.612                    | +ve     |
| IC 385912                          | 0.140                    | -ve     |
| IC 392334                          | 0.152                    | -ve     |
| Arka Sumeet                        | 0.575                    | +ve     |
| Arka Sujat                         | 0.606                    | +ve     |
| Deepthi                            | 0.806                    | +ve     |
| Coimbatore Local                   | 0.141                    | -ve     |
| Notchimedu Local                   | 0.634                    | +ve     |
| UP Variety Local (var: 100)        | 0.138                    | -ve     |
| UA 040                             | 0.452                    | +ve     |
| UA 050                             | 0.416                    | +ve     |
| SG 020                             | 0.422                    | +ve     |
| SG 030                             | 0.193                    | -ve     |
| K 070                              | 0.432                    | +ve     |
| K 090                              | 0.183                    | -ve     |
| IC 413592                          | 0.124                    | -ve     |
| IC 373361                          | 0.126                    | -ve     |
| IC 362481                          | 0.149                    | -ve     |
| IC 393014                          | 0.519                    | +ve     |
| IC 393016                          | 0.594                    | +ve     |
| IC 413577                          | 0.120                    | -ve     |
| IC 413587                          | 0.516                    | +ve     |
| LA 1                               | 0.173                    | -ve     |
| LA 2                               | 0.513                    | +ve     |
| 2S 134                             | 0.675                    | +ve     |
| CO 1                               | 0.489                    | +ve     |
| CO 2                               | 0.459                    | +ve     |
| PKM – 1                            | 0.576                    | +ve     |
| Cowpea (control)                   | 0.613                    | +ve     |
**Fig. 1** Performance of cucumber mosaic virus (CMV) disease severity in thirty five ridge gourd genotypes

**Plate 1.** Symptoms of *cucumber mosaic virus* (CMV) in different host plants by artificial sap inoculation method

a) Cowpea – Healthy plant (Control)  
Cowpea – Inoculated
Plate 2. Symptoms of *Cucumber mosaic virus* (CMV) infected plants in a) Ridge gourd and b) Cowpea by sap inoculation method.
Plate 3. Artificial *cucumber mosaic virus* (CMV) sap inoculation at 3–4 leaf stage of 35 ridge gourd genotypes

Plate 4. Post inoculation stage of ridge guard genotypes against *cucumber mosaic virus* (CMV)

Thirty five ridge gourd genotypes obtained upon mechanical (artificial) inoculation were evaluated for CMV disease severity by using DAS–ELISA method. The readings were noticed, after one hour of inoculation with pNpp substrate values greater than twice the $A_{405}$ of healthy control considered as positive (+ve) and reading below it was considered as negative (-ve) (Table 1 and Figure 1).

Thirty five ridge gourd genotypes obtained upon mechanical inoculation for CMV disease under controlled condition and were evaluated by using DAS-ELISA with control host plant of cowpea. The susceptible genotype OD readings was observed as greater than twice the $A_{405}$ of healthy control and considered as positive (+ ve) and reading below it was considered as negative (-ve). Among 35 ridge gourd genotypes only 12 genotypes of IC 92685 (0.139), IC 385912 (0.140), IC 3922334 (0.152), Coimbatore local (0.141), UP variety local (var:100) (0.138), SG 030 (0.193), K 090 (0.183), IC 413592 (0.124), IC 373361 (0.126), IC 362481 (0.149), IC 413577 (0.120) and LA 1 (0.173) had lesser OD values than control cowpea sample (0.613) and were found to be negative for CMV also considered as phenotypically tolerance. These genotypes were completely symptomless during mechanical inoculation with leaves showing CMV symptoms.
Use of disease resistant crop varieties is regarded as an economical and durable method for controlling plant diseases, especially those caused by viruses. Recently the role of mineral metabolism and total soluble phenols in imparting resistance / susceptibility against viral diseases of plants had also been manifested (Ashfaq et al., 2014). A good deal of research work has been directed to identify resistant sources under diverse environmental conditions and screening of available genotypes and new germplasm, which constitutes the basis of this work suggested by several research workers (Bashir et al., 2005; Ashfaq et al., 2007; Ashfaq et al., 2008; Ashfaq et al., 2014).

From this study, it could be concluded that, these genotypes showing resistance to cucumber mosaic virus could serve as a potential source for resistance in breeding programme and the local isolate should be needed to maintain for further studies for locating resistance sources under field conditions and for genetic manipulations and breeding purpose. But the main drawback behind the variation exists between the resistance and susceptibility of genotypes among the locations (Ashfaq et al., 2007). Therefore, environment – genotypes interaction should also be studied for durable resistance in further confirmation of tolerant ability of the genotypes (Ashfaq et al., 2008).

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