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Partial Characterization of Zucchini yellow mosaic virus infecting Gherkin (*Cucumis anguira L.*) in chittoor district of Andhra Pradesh, India.

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ABSTRACT

Gherkin (*Cucumis anguira L.*) is a vegetable crop, creeping vine that bears cylindrical fruits, carried out for exporting. A survey was carried out in 2016 to confirm the zucchini yellow mosaic viruses (ZYMV) infecting Gherkins are present in fields. ZYMV infected Gherkin samples were collected from different fields in Shantipuram, Ramakuppam, Kuppam, Srikalahasti mandal villages in Chittoor district of Andhra Pradesh. Infected plants exhibit severe stunting of plant, yellow mosaic, blistering on leaves and the fruit deformation was observed. The collected samples were tested by DAC-ELISA by using different antisera, it was reacted positively with polyclonal antiserum of Potyvirus. ZYMV belongs to potyviridae family, have a genome of positive single stranded RNA, non-enveloped, flexuous filamentous rods, the coat protein is a single polypeptide. ZYMV infected young Gherkin leaves were collected and grinded with 0.01M potassium phosphate buffer and the extract was mechanically inoculated to cotyledons of young Pumpkin seedlings. These virus isolates were maintained in pumpkin seedlings which were kept in earthen pots at insect proof glass house. ZYMV infected Gherkin was purified by PEG method, followed by Sucrose density gradient centrifugation. The coat protein molecular weight was determined by using SDS-PAGE was estimated at 32KDa.

Keywords: Gherkin yellow mosaic virus, purification, polyclonal antibodies, ELISA

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INTRODUCTION

Cucumis anguira L (Family: Cucurbitaceae) mainly grow for its crispy, moisture rich flesh, small edible fruit. It is used for make chiefly slicing, pickles and specially for exporting. A number of viral diseases affect cucurbits, major of them are Squash leaf curl virus (SLCV), watermelon chlorotic stunt virus (WmCSV), Cucurbit yellow stunt disorder virus (CYSDV), Cucumber mosaic virus (CMV), Cucurbit aphid borne yellow virus (CABYV), Moroccan watermelon virus (MWmV), Papaya ring spot virus (PRSV), Watermelon mosaic virus (WMV), Zucchini yellow mosaic virus (ZYMV) has been reported. Among these ZYMV is most wide spread plant viral pathogen affecting cucurbitaceae.

ZYMV was determined and found to have the features that are characteristic member of the family Potyviridae. The ZYMV genome is 9593 nucleotides long (Galon et.al 2007), positive single stranded RNA with 5^1 viral protein genome linked (VPg) and 3^1poly (A) tail encapsidated in 32 KDa coat protein (Shukla et.al,1994). In fields, symptoms on Gherkin plants are commonly very severe and induce significant yield reduction. Leaves show a severe yellow mosaic, blistering of leaf lamina, leaf deformation was observed. Infected fruits exhibit severe deformation and colour alteration, distorted with prominent lumps. Aphids can transmit the virus in non-persistent manner. Host range of ZYMV includes *Cucumis melo*, *Cucumis anguria, Cucumis sativus*, *Cucurbita pepo, Cucurbita moschata, Cucurbita maxima* and *citrullus lanatus*etc. (Lee et.al, 2007). The objective of the present study is to purify the zucchini yellow mosaic virus from ZYMV infected Gherkin leaves and further characterization of the virus.

MATERIALS AND METHODS

Zucchini yellow mosaic virus infected Gherkin leaf samples were collected from various regions across Shantipuram, Ramakuppam, Kuppam, Srikalahasti mandal villages in Chittoor district of Andhra Pradesh. The collected samples which are systemically infected were tested by DAC-ELISA with different antiserums. Infected Gherkin leaves were homogenised in chilled 0.01M potassium phosphate buffer and mechanically inoculated on the carborandum dusted cotyledons of pumpkin which was used as a propagation host. Inoculated plants were kept in an insect proof glass house to allow the development of symptoms. Purification of virus was done by polyethylene glycol method (Prieto et.al, 2001).

ZYMV were purified from 100g of symptomatic leaf samples. The leaf samples were grounded in liquid nitrogen and suspended in 200 ml of 0.1M potassium phosphate buffer (PH-8.0) containing EDTA, Sodium sulphite and 2 mercaptoethanol (Lisa et.al, 1981). It was filtered through a double layered muslin cloth. The initial extract was emulsified with 10% chloroform for 15-20 minutes. This was subjected to centrifugation at 10,000 rpm for 15 minutes. After centrifugation, to the supernatant 20% PEG was added and allow to stir for 45 minutes (Huang et.al,1986). After stirring allow it in 4ºC for 90 minutes. After 90 minutes of incubation it was subjected to centrifugation for 15 minutes at 10,000rpm. Supernatant was discarded and the pellet was dissolved in 0.01M potassium phosphate buffer and kept in 4ºC for overnight. The resulting emulsion was centrifuged at 8000 rpm for 5 minutes; the upper aqueous phase was collected and layered over 10-40% linear sucrose density gradients followed by centrifugation at 27,000 rpm for 2.30 hours. After spin the light scattering zone was collected and it was dissolved in 1:1 ratio of 0.01M potassium phosphate buffer and centrifugation at 30,000 rpm for 3 hours. The final pellet was suspended in 0.01M potassium phosphate buffer. All the steps are done at 4ºC only. Coat protein molecular weight was determined by SDS-PAGE (Laemmli et.al, 1970)

Healthy New Zealand white Rabbit was taken for immunization. 1 mg of purified virus was emulsified with an equal volume of complete Freunds adjuvant and injected into the thigh muscle of the rabbit. Four injections were given at one week interval with incomplete freunds adjuvant. Seven days after the last injection, the rabbit was test bleed by making a cut on the marginal ear vein with a sharp sterile blade. Blood was allowed to clot for at 4ºC. The tubes were centrifuged at 6000 rpm for 15 minutes and antiserum was collected into 1 ml aliquots and stored in deep freezer (-20ºC). DAC-ELISA (Hobbs et.al, 1987) was performed to determine the titre of the above raised antibodies against ZYMV.

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RESULTS AND DISCUSSION

Zucchini yellow mosaic virus infected Gherkin leaf samples collected from Shantipuram, Ramakuppam, Kuppam, Srikalahasti mandal villages in Chittoor district during cropping season. ZYMV infected Gherkin leaves showing severe yellow mosaic, blistering of leaf lamina, vein banding, leaf reduction, mild to severe mottling symptoms were observed **(Fig.1).**

Fig 1: Gherkin leaves showing severe yellow mosaic; blistering of leaf lamina, vein banding, leaf reduction, mild to severe mottling symptoms

The infected fruits exhibit severe deformation with lumps was occurred. The collected leaf samples were tested by DAC-ELISA using different antiserums (provided by Department of Virology) it was strongly reacted with homologous polyclonal antiserum of potyvirus. The positively reacted ZYMV leaf samples were propagated on 3-4 leaf stage pumpkin seedlings which was mechanically sap inoculated and maintained on insect proof glass house.

ZYMV was purified from infected Gherkin leaves. The purified virus obtained shows a maximum absorption at 10.160 and minimum absorption at 7.255 at 240 nm. It's A_{260}/A280 ratio was 1.40 (Brunt et.al, 1990). The virus yield was ranged from 2.1mg/ 100 g of freshly infected Gherkin leaves.

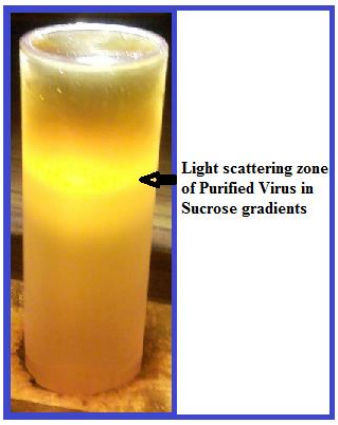

Fig 2: Light scattering zone of purified virus

Purified virus was proved to be infectious on pumpkin when inoculated on healthy seedlings. SDS-PAGE analysis revealed the molecular weight of virus coat protein approximately 32 KDa in size of purified virus preparation.

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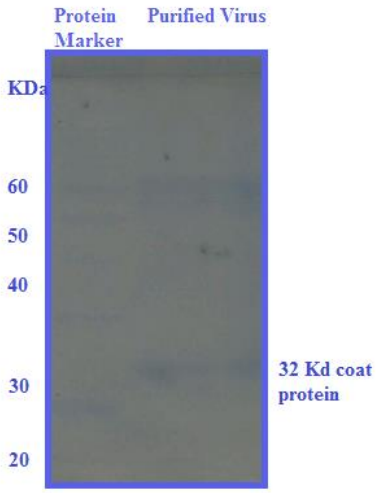

Fig 3: SDS PAGE analysis of coat protein of virus

After five successive injections of the purified virus the test bleed was done by cutting the vein on ear of the rabbit and the serum was collected. Antiserum was collected at weekly intervals and the titre was detected by performing DAC-ELISA. The positive reaction up to 1/5000 titre of antisera was observed (Fig.4). No reaction was observed in healthy and buffer control.

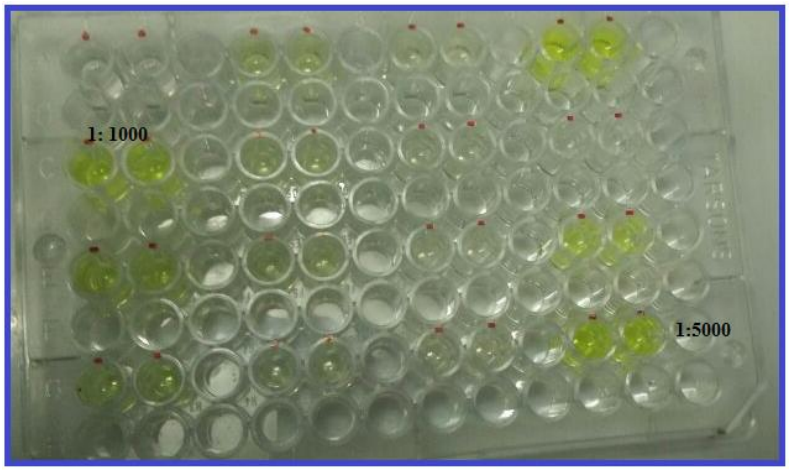

Fig 4: DAC ELISA of polyclonal antibodies

CONCLUSION

Gherkin is one of the important crops of cucurbitaceae grown in southern states of India. During field survey stunting of plants, yellow mosaic, blistering of the leaves and fruit deformation was observed. Both the natural symptoms on Gherkin and artificial induced symptoms on Pumpkin seedlings were different. In DAC ELISA with potyvirus antiserum it was reacted positively. Purification of ZYMV from infected Gherkin leaves were no one did so far. But in this present study ZYMV was purified from Gherkin leaves and concentration was found to be good and the purified virus was seen as single scattered zone in Sucrose gradient centrifugation. From purified virus SDS PAGE the coat protein molecular weight at 32 KDa as like potyvirus.

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