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Seed Borne Nature of Begomoviruses Infecting Bitter gourd in Tamil Nadu

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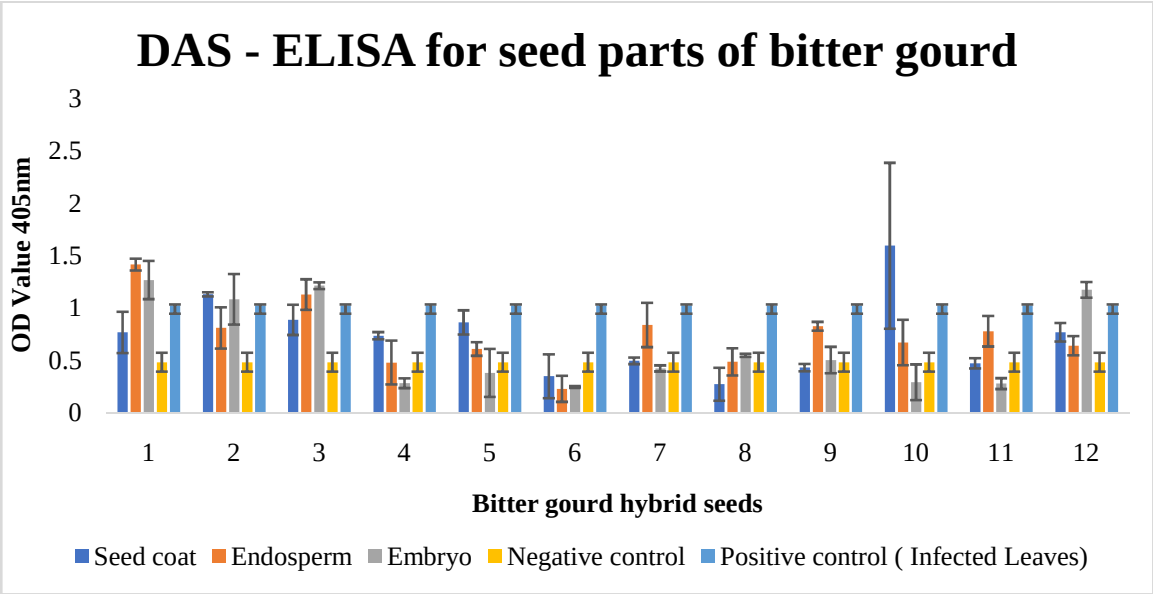
ABSTRACT: Bitter gourd is a major cucurbitaceous crop that serves as a natural host for many viruses. Especially in recent decades, the crop was severely affected by begomoviruses such as tomato leaf curl New Delhi virus and Bitter gourd yellow mosaic virus (ToLCNDV & BgYMV) in various regions of country. The presence and amount of virus titre in different parts of seeds of a predominant hybrid grown by the farmers was analysed by DAS – ELISA. The virus titre is high in endosperm 0.22 to 1.86 followed by seed coat and embryo with OD values of 0.27 to 1.59 and 0.24 to 1.50 respectively. The presence of virus in the embryo is the major criterion for the successful transmission of the begomoviruses to the progeny which results in a rapid and high infection rate and also serves as an inoculum source for transmission by whiteflies.

Keywords: Bitter gourd, seeds, begomoviruses, DAS – ELISA.

INTRODUCTION

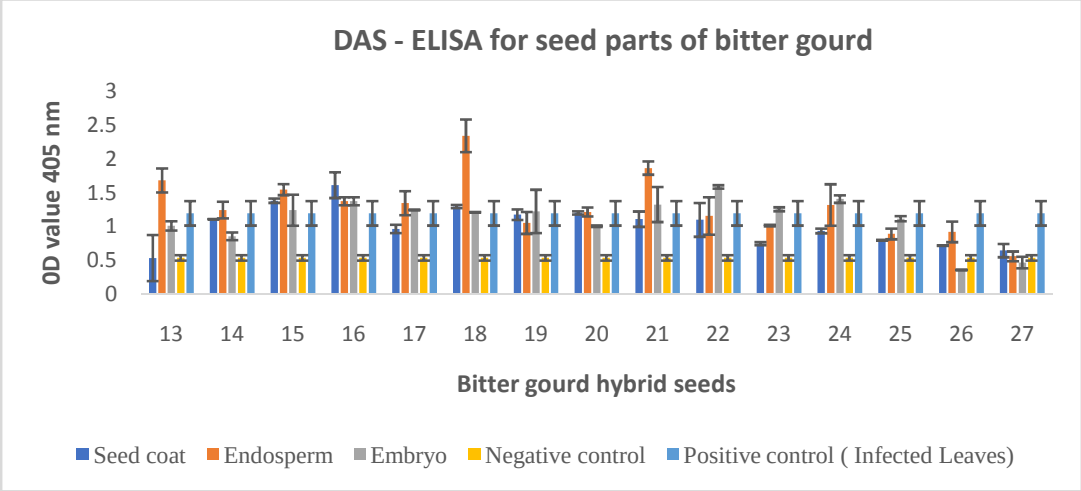
Bitter gourd (*Momordica charantia* L), also referred as bitter cucumber, balsam pear, bitter melon, bitter squash belongs to the family *Cucurbitaceae*. It is an important vegetable in south Indian states originated in Indo-Burma region. Recently, continuous cultivation of bitter gourd resulted in serious yield loss due to numerous fungal, bacterial, and viral illnesses. The important viral diseases, reported in bitter gourd includes papaya ring spot virus (Chin *et al*., 2007; González Vera *et al*., 2003; Ohtsu, 1988), cucumber mosaic virus (Takami *et al*., 2006), watermelon silver mottle virus (Tokashiki, 1991), zucchini yellow mosaic virus (Ohtsu, 1988), watermelon mosaic virus-1 (Tomar and Jitendra 2005), Indian cassava mosaic virus (Rajinimala *et al*., 2005), Cucurbit leaf crumple virus and Squash vein yellowing virus (Adkins *et al*., 2008), Melon yellow spot virus (Takeuchi *et al.*, 2009), Pepper leaf curl Bangladesh virus (Raj *et al.*, 2010) . In India bitter gourd has been plagued by severe yellow mosaic disease for the past decade. The symptoms such as yellowing, leaf lamina distortion, puckering, and stunting are produced and found to be transmitted by whitefly *Bemesia tabaci* (Fig. 1). Tomato leaf curl New Delhi virus, Ageratum enation virus (AEV), Squash leaf curl China virus (SLCCNV), Coccinia mosaic Tamil Nadu virus (CoMoV), Tomato leaf curl Palampur virus (ToLCPalV) and Bitter gourd yellow mosaic virus are the six important begomovirus species discovered which are linked to different cucurbitaceous crops (Manivannan *et al.*, 2018).

Fig. 1. Mosic mottling symptom of begomovirus infection in bitter gourd.



Bars represent the mean for three replications and errorbars represent the standard error for mean value.

Fig. 2. Graphical representation of virus titer in terms of OD value in different seed parts of bitter gourd (1-12 seeds).



Bars represent mean for three replications and errorbars represent the standard error for mean value.

Fig. 3. Graphical representation of virus titer in terms of OD value in different seed parts of bitter gourd (13-27 seeds).

Begomoviruses are non-enveloped viruses with twinned (geminate) para icosahedral particles measuring 22×38 nm in diameter and 2.5-3 kb of single-stranded circular DNA. Their genomes are either monopartite or bipartite. On the basis of hosts, vectors and genome organization, fourteen genera are differentiated within the family; the genera are *Becurtovirus*, *Begomovirus*, *Capulovirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus* *Turncuurtovirus*, Citlodavirus, Maldovirus, Mulcrilevirus, Opunvirus and Topilevirus. Bidirectional transcription occurs in circular single-stranded DNA, with coat protein encoded on the sense strand and replication initiation protein on the complementary strand (Philippe *et al.*, 2021)

Begomoviruses were formerly thought not to be seed transmissible, because they were confined to phloem tissue. The reports of seed transmission of begomoviruses such as Sweet potato leaf curl virus (SPLCV) (Kim *et al.*, 2015), Tomato yellow leaf curl virus (TYLCV) (Kil *et al.*, 2016), Mungbean yellow mosaic virus (MYMV) (Kothandaraman *et al.*, 2016), Tomato leaf curl New Delhi Virus (ToLCNDV) (Manivannan *et al.*, 2019), Dolichos yellow mosaic virus (DoYMV) (Suruthi *et al.*,2018), Sweet potato symptomless virus 1 (SPSMV-1) (Qiao *et al.*, 2020),

Pepper yellow leaf curl Indonesia virus (PepYLCIV) (Fadhila *et al*., 2020) contradicts with earlier reports.

In the case of bitter gourd, Bitter gourd yellow mosaic virus (BgYMV) was reported to be seed transmissible with seed infectivity range of 79.16% and the transmission rate to seedling was 32.05% (Manivannan *et al*., 2018). In the fields surveyed during (2016 – 2017), the disease incidence was 100%, and the fruits were severely deformed, lowering the marketable yield. The present study investigates the virus titre in different parts of bitter gourd hybrid seeds by DAS – ELISA, which will provide information about seed borne nature of begomovirus in hybrid seeds which is widely grown by farmers.

MATERIALS AND METHODS

Hybrid seeds of bitter gourd purchased from market were subjected to double antibody sandwich – Enzyme linked immune sorbent assay DAS-ELISA (Clark and Adams, 1977). Totally 27 seeds as three different parts viz., seed coat, endosperm, embryo and 7 whole seeds were tested. In this study, healthy bitter gourd plants grown under insect proof conditions were included as negative control, which were tested negative for Roja's primer. Symptomatic leaves from the field were collected and used as a positive control. The presence of begomovirus was confirmed using DAS-ELISA (Swanson *et al.,* 1992). Microtitre plates with 96 wells were used to perform DAS-ELISA utilising ToLCNDV polyclonal antibody (DSMZ, DAS ELISA kit, cat. no. # AS-1109) (Micro Test Plate Flat bottom, Tarson Pvt. Ltd, Kolkata, India). ToLCNDV IgG (AS1109) was diluted to 1:1000 in coating buffer and100µl of diluted antibody was added into individual wells of ELISA plate and incubated at 37°C for 4 hours. Plates were washed thrice with PBS-T at 3 min interval. The seeds were soaked overnight and the seed parts were separated as seed coat, endosperm, embryo and whole seeds were ground thoroughly in a sterile pestle and mortar with general extraction buffer (50 mM Tris, 10 mM EDTA, pH 8.0, 2% PVP) at 1 ml/g of sample. Each sample was replicated thrice. The extract was allowed to sit on ice so that debris will settle on bottom and clear aqueous will be obtained. 100 µl of extract prepared from each sample was added into the individual wells of ELISA plate and incubated at 4 °C overnight. Plates were washed thrice with PBS-T at 3 min interval. 100 µl of IgG enzyme conjugate (AS1109 IgG – AP) diluted at 1:500 dilution in Enzyme conjugate buffer was added to each well and incubated at 37°C for 4 hours.100 µl of p-nitro phenyl phosphate (p-NPP) substrate (Sigma Aldrich, USA) was added to each well and incubated in dark condition for 30 min. For each sample, three replications were maintained. Optical density (OD) was recorded at 405 nm 1 hr after the addition of substrate. Absorbance value which is twice more than the values obtained in the healthy control were considered as a positive reaction.

RESULTS

In DAS ELISA conducted for whole seeds, all the seven seed samples were positive with OD value of 1.08, 1.65, 1.75, 2.02, 1.00, 1.05 and 1.42 as compared to negative control (0.48). In the case of seed parts tested, among 27 samples tested, 10 seed coats were positive with OD value of 1.10 - 1.61, 14 endosperms were positive with OD value of 1.05 - 1.86, among embryo samples, 17 were positive with OD value ranged from 1.08 - 1.50 as compared to negative control (0.54). Among different seed parts, endosperm recorded the highest OD value than seed coat and embryo (Table 1).

DISCUSSION

Cucurbits, are widely grown in India for their medicinally valuable, nutrient-rich fruits. Due to its significance in medicine, bitter gourd is the most popular of all the cucurbits. Since 2009, Yellow mosaic disease caused by begomoviruses resulted in 100% yield loss. The seed borne and seed transmission nature of begomoviruses constitutes the important factor for active spread of diseases within the field. Manivannan *et al*. (2018) first reported the seed borne nature of begomovirus in bitter gourd by detecting BgYMV in different seed parts of about 79.16%. He reported that among five seeds dissected in to three parts, seed coat revealed high concentration of bitter gourd yellow mosaic virus (BgYMV) followed by endosperm and embryo and among 24 whole seeds tested 19 were positive. Kil *et al*. (2021) reported that the seed coats of Zucchini squash and emerged seedlings were positive in PCR analysis for tomato leaf curl New Delhi virus (ToLCNDV). In our study, among 27 seeds dissected in to three parts *viz.,* seed coat, endosperm and embryo, endosperm recorded high concentration of begomovirus followed by seed coat and embryo and all the seven whole seeds tested were positive and revealed high concentration of begomovirus. Since the study was conducted with polyclonal antiserum of ToLCNDV, it reacted for all the begomoviruses involved. Thus, past reports and current results revealed the presence of begomovirus in embryo, which plays a key factor for seed transmission from one generation to other generation. Further the begomoviruses involved are to be confirmed by rolling circle amplification and PCR studies.

Table 1: Detection of begomovirus in different seed parts and whole seeds of bitter gourd through DAS-ELISA.

	OD at 405 nm*			
Seed Number	**Seed coat**	**Endosperm**	**Embryo**	**Whole seeds**
1.	0.71±0.19	**1.41±0.05**	**1.26±0.18**	**1.08±0.25**
2.	1.12±0.01	0.80±0.19	1.08±0.24	1.65±0.12
3.	0.88±0.14	1.12±0.14	1.21±0.03	1.75±0.19
4.	0.73±0.34	0.47±0.20	0.28±0.04	2.02±0.48
5.	0.85±0.11	0.60±0.06	0.37±0.22	1.00±0.16
6.	0.34±0.2	0.22±0.12	0.24±0.00	1.05±0.05
7.	0.49±0.03	0.83±0.21	0.42±0.02	1.42±0.05
8.	0.27±0.15	0.48±0.12	0.54±0.01	PC - 1.02±0.00
9.	0.42±0.03	0.82±0.04	0.50±0.12	NC - 0.48± 0.00
10.	**1.59±0.79**	0.66±0.21	0.28±0.17	
11.	0.47±0.04	0.77±0.14	0.27±0.05	
12.	0.76±0.08	0.63±0.09	**1.17±0.07**	
13.	0.53±0.34	**1.68±0.17**	**1.01±0.06**	
14.	**1.11±0.00**	**1.24±0.12**	**1.12±0.01**	
15.	**1.38±0.03**	**1.55±0.08**	**1.24±0.22**	
16.	**1.61±0.19**	**1.37±0.59**	**1.37±0.05**	
17.	0.96±0.06	**1.35±0.17**	**1.24±0.00**	
18.	**1.29±0.02**	**2.34±0.24**	**1.21±0.00**	
19.	**1.17±0.07**	**1.05±0.16**	**1.22±0.32**	
20.	**1.20±0.02**	**1.21±0.06**	**1.00±0.01**	
21.	**1.11±0.11**	**1.86±0.09**	**1.32±0.25**	
22.	**1.10±0.24**	**1.15±0.27**	**1.50±0.79**	
23.	0.74±0.02	**1.01±0.01**	**1.26±0.18**	
24.	0.93±0.03	**1.32±0.30**	**1.41±0.05**	
25.	0.80±0.00	0.89±0.07	**1.12±0.14**	
26.	0.72±0.00	0.92±0.15	0.36±0.00	
27.	0.64±0.09	0.56±0.07	0.47±0.08	
Positive control	1.20±0.18			
Negative Control	0.54±0.00			
Buffer control	0.03±0.00			

*Value represents the average of three replications. Value twice that of the negative control is considered as positive, which is given in bold.

CONCLUSION

The study strongly infers the seed borne nature of begomoviruses infecting bittergourd and virus detection in embryo of hybrid seeds indicates that there is maximum chance of seed transmission. An in-depth analysis is pre request to produce virus free seeds.

FUTURE SCOPE

A successful seed treatment approach can be developed to produce seeds that are virus-free despite the presence of the virus in bitter gourd hybrid embryos.

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Conflict of Interest. None.

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