International Journal of Plant & Soil Science

Volume 36, Issue 2, Page 292-302, 2024; Article no.IJPSS.111624
ISSN: 2320-7035

Distribution of Mosaic Disease in Ridge Gourd (*Luffa acutangula* (L.) Roxb.), Characterization of Associated Virus and Screening for Virus Resistance

T. Sana [a], Radhika N. S. [b*], Sajeesh P. K. [a], Reshmika P. K. [c], Sainamole Kurian P. [a] and Shameer Mohammed E. [d]

[a] *Department of Plant Pathology, College of Agriculture, Padannakkad, Kerala Agricultural University, Thrissur, 671314, India.*
[b] *Department of Plant Pathology, College of Agriculture, Vellayani, Kerala Agricultural University, Thrissur, 695522, India.*
[c] *Plant Propagation and Nursery Management Unit, Vellanikkara, Kerala Agricultural University, Thrissur, 680656, India.*
[d] *Department of Soil Science and Agricultural Chemistry, College of Agriculture, Padannakkad, Kerala Agricultural University, Thrissur, 671314, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2024/v36i24391

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/111624

Original Research Article

Received: 07/12/2023
Accepted: 28/01/2024
Published: 02/02/2024

ABSTRACT

Aims: A survey was conducted in the ridge gourd growing areas of Kasaragod, Kannur and Kozhikkode district, India, to assess the prevalence of virus diseases. Molecular detection and characterization indicated the presence of mixed infection of *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Cucumber mosaic virus* (CMV) in infected samples of ridge gourd collected from

**Corresponding author: E-mail: Radhika.ns@kau.in;*

surveyed locations. Additionally, a screening of popular varieties/hybrids was undertaken to identify resistance sources against the Begomovirus in Ridge gourd.
Study Design: CRD design for screening for resistance genotype.
Place and Duration of Study: Department of Plant Pathology, College of Agriculture, Padannakkad. November 2022 - 2023.
Methodology: Twenty-five ridge gourd plants were selected from a field and scored during survey. Major weeds and insects in and around the location were collected and identified. Screening for resistance was carried out *in vitro* by grafting infected scion on to healthy ridge gourd plants. Begomovirus infections were confirmed by polymerase chain reaction (PCR) using specific coat protein primers. Presence of CMV was detected using specific primers.
Results: Disease incidence (DI) of surveyed locations ranged from 5.3-100 per cent and vulnerability index (VI) from 26.57-84.08. Kunjathur, Thalangady, and Hosabettu areas of Kasaragod district recorded the highest incidence (100%). The highest VI (84.08) was recorded in Thalangady (Kasaragod). *Bemisia tabaci* and *Aphis gossypii* were the associated insects and *Synedrella* sp., *Ageratum conizoides* and *Alternanthera* sp. were the associated weeds.
Screening of varieties indicated moderately susceptible reactions of RG7 and RG10 after 60 days of grafting. PCR based molecular detection of ToLCNDV using coat protein specific primers yielded amplicons of approximately 575 bp. All the isolated sequences were found to be related to ToLCNDV on sequencing and blast analysis. Phylogenetic studies revealed that isolates from Thrikaripur, Mavichery, and Poolacode were closely related to each other. *Cucumber mosaic virus,* a RNA virus was also detected from isolated samples using specific primers of CMV with an amplicon size of 400 bp.
Conclusion: The study revealed that the ridge gourd cultivated in areas of Kasaragod, Kannur and Kozhikode were infected with ToLCNDV and CMV; RG7 and RG10 were moderately susceptible among the varieties and hybrids screened. The presence of the begomovirus was detected in weeds indicating its role in perpetuation and spread of the virus disease in the field.

Keywords: Ridge gourd; survey; ToLCNDV; CMV; PCR.

1. INTRODUCTION

Cucurbit crops are significant economic contributors for farmers and small-scale landowners. Over 4,479,058 tons of cucurbit crops have been harvested from 240,603 acres of land throughout Southeast Asia [1]. *Luffa acutangula* (L.) Roxb., often known as angled loofah, angled gourd, or ridge gourd, is a subtropical Asian vegetable and whose center of origin is regarded as India [2]. Ridge gourd fruits imparts therapeutic properties such as antifungal, antioxidant, antidiabetic in addition to nutritional qualities (1.21-1.58 per cent soluble sugar, 14.5-36.1 mg/100g carotenoid, 0.175-0.253 per cent protein, 1.59-1.85 mg.g-1 chlorophyll). In addition, fruits are rich in phytin, flavonoids, saponin and vitamins [3].

Even though production efficiency of ridge gourd is high, yield loss due to diseases and pests attack is significantly high. Major biotic constraints caused by viruses are emerging as a potential threat to the cultivation. More than 70 different viral species are reported to be causal agents of various infections in cucurbits [4]. *Tomato leaf curl New Delhi virus* (ToLCNDV) [5,6], *Watermelon bud necrosis virus* (WBNV) [7], *Cucumber mosaic virus* (CMV) [8] and *Zucchini yellow mosaic virus* (ZYMV) [9] are the main infectious agents reported in ridge gourd. Among all these, Begomovirus, largest genera in the family Geminiviridae are becoming major issues in India for numerous cucurbits [10,11]. Begomoviruses infected ridge gourd plants exhibited mosaic with light chlorotic region on the leaf lamina, upward curling, blisters on leaves, reduction in leaf size, yellowing, cupping and at severe infection, stunting of ridge gourd plants [6]. It is widely spread to different geographical area through vectors like *Bemisia tabaci*. Timely detection and diagnosis are essential to overcome the losses caused by virus infection. Recently, the incidence of virus diseases has reduced the enthusiasm of cultivation in northern districts of Kerala. Scientific knowledge regarding the viruses affecting this crop, molecular detection and relationship of this virus with other already reported ones in cucurbits are sparse; moreover, Identifying the sources of resistance if the cheapest method of management of virus infection and hence this study was undertaken.

2. MATERIALS AND METHODS

2.1 Survey

The survey for virus disease incidence in ridge gourd was conducted in major vegetable growing areas of Kasaragod, Kannur and Kozhikode (AEU 2 and 11) districts of Kerala (Fig. 1). Three locations in each taluk and two taluks of each district were chosen for study. In total, 19 ridge gourd fields were examined for the prevalence of viral infections (Table 1). Twenty-five plants were randomly selected from a field and scored on a 0–5 scale [12] (Fig. 2). Major weeds and insects were collected and identified. Total of six ridge gourd plants collected from each taluk surveyed and two weeds were detected with the presence of Begomovirus and CMV by PCR.

0-No symptom
1-Very light mottling
2-Mottling with dark green and yellow colour
3-Blisters and raised surface on leaves
4-Distortion of leaves, yellowing, curling, hairiness, reduction in leaf size
5-Stunting with negligible or no flowering and fruiting or very small fruits

For each field surveyed, DI and VI were calculated.

$$DI = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ plants} \times 100$$

$$VI = \frac{(0n0+1n1+2n2+3n3+4n4+5n5)}{nt\ (nc-1)} \times 100$$

where:

$n1$,$n2$,..$n5$ = Number of leaves in a selected plant in disease category 1,2,….5 respectively
nt = Total number of leaves selected for scoring per plant
nc= Number of categories

2.2 Screening for Resistance

Seeds of popular cultivated varieties/hybrids of ridge gourd, KAU released variety/hybrid were collected and raised in pots under greenhouse condition to screen the resistance against viral infection. Ridge gourd scions infected with Begomovirus were procured from Manjeshwar in Kasaragod district on the day of grafting. A wedge- or V-shaped cut was made on healthy rootstock at 4-5 leaf stage, right above the third leaf. Scion was prepared by slant cut to paper thickness. Root stock and scion was joined by grafting clips. A PVC pipe of 25 cm length and 6 cm diameter was used to cover grafted seedling for two days and its open mouth was covered with a muslin fabric in order to get humidity for graft union. Symptom expression on new flushes of rootstock was recorded from fifth day and continued up to eight weeks to check the disease progress and detected molecularly for the presence of Begomovirus.

2.3 Molecular Detection, Characterization and Phylogenetic Analysis

2.3.1 Isolation of genomic DNA and confirmation by agarose gel electrophoresis

Invitrogen Genejet plant DNA extraction kit was used to isolate total plant genomic DNA from ridge gourd, *Synedrella* sp. and *Alternanthera* sp. according to the manufacturer's protocol. Agarose gel (0.8 %) was prepared and 3 µl ethidium bromide, an intercalating agent, was added to it and casted in horizontal gel casting tray (Cleaver Scientific). 4 µl DNA mixed with 1 µl of 6X loading dye to track the DNA and loaded in each well. One kb DNA ladder (Invitrogen TrackIt) was loaded in first well to recognize the size of total genome. The loaded gel was run in electrophoresis unit (Cleaver Scientific) for 55 minutes in 1X TAE buffer at 80 V cm-1.

2.3.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction **(**PCR) was carried out in SimpliAmp Thermal Cycler to amplify the isolated total genomic DNA of ridge gourd and weeds using specific molecular primers of Geminivirus group viz., AV/ACprimers(AV494-F-GCCHATRTAYAGRAAGCCMAGRAT, AC1048-R- GGRTTDGARGCATGHGTACANGCC) [13]. The primers used for amplification were diluted to 10µM with DNase and RNase free water. 7µl 2X PCR mastermix (TaKaRa) with loading dye (VWR Lifesciences), 0.5 µl forward primer, 0.5 µl reverse primer, 3 µl DNase and RNase free water,and 1.5µl isolated DNA were taken in the PCR tube and kept a total of 12.5µl volume for amplification. Specific annealing temperature was standardized in case of ridge gourd, *Synedrella* sp. and *Alternanthera* sp. (Initial denaturation- 94°C for 1 minute- one cycle, denaturation- 94°C for 50 seconds, primer annealing- 52°C for 45 seconds, primer extension- 72°C for 2 minutes- 35 cycle, final extension- 72°C for 10 minutes – one cycle) to get complete amplification in respective cycles.

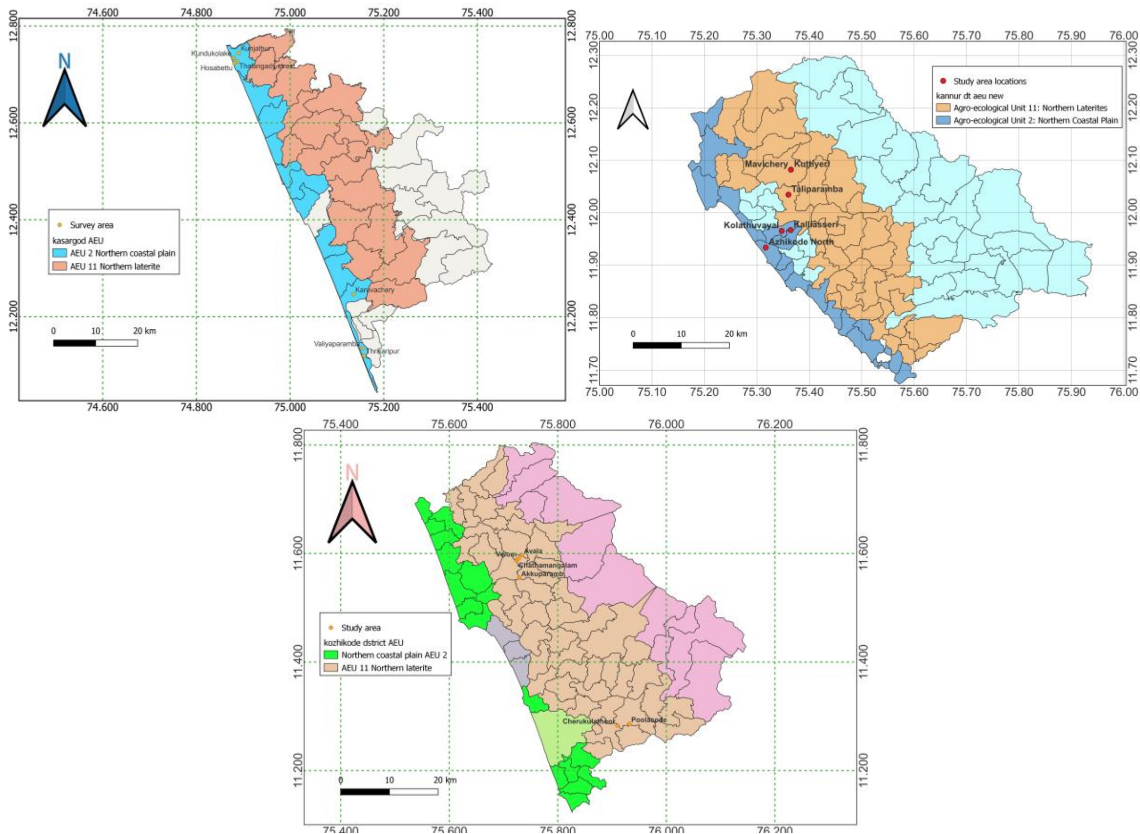

Fig. 1. Surveyed locations (Location map) in Kasaragod, Kannur and Kozhikkode districts

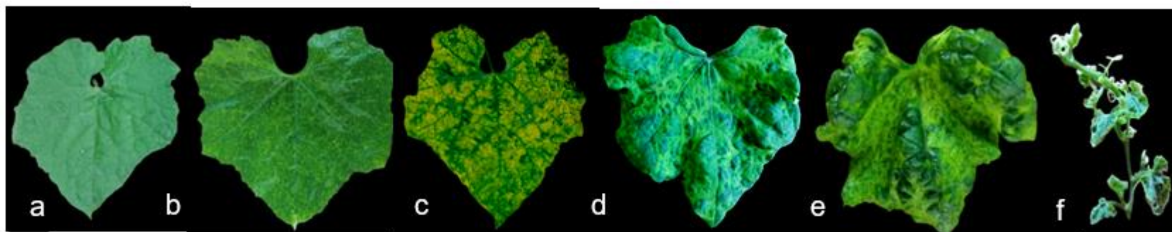

Fig. 2. Score chart (0-5 scale) for virus disease infecting ridge gourd a) 0- No symptom b) 1- Very light mottling c) 2- Mottling with dark green and yellow colour d) 3- Blisters and raised surface on leaves e) 4- Distortion of leaves, yellowing, curling, hairiness, reduction in leaf size f) 5- Stunting with negligible or no flowering and fruiting or very small fruits

2.3.3 Agarose gel electrophoresis of PCR products

1.2 % agarose gel was casted on horizontal casting tray (Cleaver Scientific) after adding 3µl Ethidium bromide. 6 µl of PCR product was directly loaded in each well keeping first well for 3µl 1Kb DNA ladder (Invitrogen TrackIt). By applying 80 V cm^{-1} gel was allowed to run in 1X TAE buffer for 70 minutes. The gel was taken out and placed in Gel documentation system to visualize and document DNA bands.

2.3.4 Characterization and phylogenetic analysis

Isolated DNA from Thalangady, Thrikaripur, Mavichery, Thaliparamba and Poolacode was sequenced from Phytocom pvt. Limited, Cochin. Similarity of isolates obtained were checked with Basic Local Alignment Search TooL (BLAST) with existing database in NCBI (National Centre for Biotechnology Information). Percentage similarity between each Begomovirus was compared with multiple sequence alignment tool and most

related 15 strains are selected for construction of phylogenetic tree in MEGA 11.0 [14].

2.3.5 Isolation of genomic RNA and confirmation by Agarose gel electrophoresis

RNA was isolated from ridge gourd plant collected from surveyed location by TRIzol Reagent [15]. Isolated RNA converted into cDNA by Revert Aid First Strand cDNA Synthesis Kit of Thermo Fisher Scientific. It was then used for PCR amplification with CMV1 (5'-GCC GTA AGC TGG ATG GAC AA-3') and CMV2 (5'-TAT GAT AAG AAG CTT GTT TCG CG-3') primer under suitable condition (Initial denaturation- 94°C for two minutes-one cycle, denaturation-94°C for 20 seconds, primer annealing- 57°C for 30 seconds, primer extension- 72°C for one minute- 30 cycles, final extension- 72°C for 10 minutes-one cycle). Reaction mixture was prepared with 9 µl of cDNA, 34.75 µl PCR water, 5 µl of 10 x PCR buffer without detergents, 1 µl of 20 pM CMV1, 1 µl of 20 pM CMV2, 4 µl of 10 mM dNTP mixture at a concentration of 200 µM), 3 µl of $MgCl2+$(25 mM), 0.25 µl 5U/µl recombinant Taq DNA polymerase for PCR. Detection of RNA was done by gel electrophoresis with 1.2 percent agarose gel at 75 V cm^{-1} for one hour. Gel documentation system (Bio-rad) was used for visualization and documentation of RNA.

3. RESULTS AND DISCUSSION

3.1 Survey

The survey for viral disease incidence and vulnerability index in ridge gourd was conducted in major vegetable growing areas of Kozhikode, Kannur and Kasaragod (AEU 2 and 11) districts of Kerala during 2021-2023 (Table 1). Three locations in each taluk and two taluks of each district were chosen for study. From Kasaragod district, Thrikaripur, Valiyaparamba, Karuvachery, Kunjathur, Thalangady street, Kundukolake and Hosabettu areas were selected. Plants showing yellowing, blistering, cupping, vein banding and stunted growth were randomly selected from each location. The disease incidence was highest (100%) in Kunjathur, Hosabettu and Thalangady. Disease incidence of Kundukolake (76.47%), Valiyaparamba (39.06%), Thrikaripur (32.30%) and Karuvachery (17.85%) were also recorded. Disease reaction of leaves were scored and VI was calculated according to Bos chart [16]. Highest VI (84.08) was recorded in Thalangady region, followed by Hosabettu (77.92), Kunjathur (57.60), Karuvachery (42.64), Kundukolake (39.44), Valiyaparamba (30.56) and Thrikaripur (26.57).

In Kannur district, surveys were carried out in ridge gourd growing areas of Thaliparamba and Kannur taluk.Kuttyeri recorded highest disease incidence (90%) followed by Mavichery (76.6 %) and Thaliparamba (8.4 %). Vulnerability index recorded from Kuttyeri, Mavichery and Thaliparamba were 61.2, 43.44 and 38.29 respectively. Disease incidence of Azhikode north (26.56 %), Kolathuvayal (11.67 %) and Kalliasseri (5.3 %) were recorded and VI indicated were 40, 53.05 and 49.5 respectively.

Six ridge gourd growing areas of Kozhikkode district was surveyed. Highest disease incidence in Cherukulathur (68 %) was recorded with a VI of 34.88. Both Poolacode and Chathamangalam recorded disease incidence (35%) with 35.71 and 26.57 VI respectively. No symptomatic plants were observed in ridge gourd cultivated fields at Avala, Akkuparamb and Velom.*Alternanthera* sp., *Ageratum conizoides* and *Synedrella* sp. with mosaic and vein clearing symptom was found around the infected fields in Kasaragod, Kannur and kozhikkode districts. Whitefly and aphid were found associated in surveyed locations.

High DI in three locations of Kasaragod district might be due to year-round production of ridge gourd plant with highly susceptible variety chosen. Mixed cultivation of cucurbit crops like bitter gourd, pumpkin, ash gourd, ridge gourd, oriental pickling melon and bhindi, cassava in same field may be the reason for high disease incidence and severity in Kuttyeri region of Kannur. This leads to higher crop density, favourable microclimate for vectors hence easier vector transmission of virus. High population of weed plants were present in and around the fields of Kozhikkode, Kasaragod and Kannur area, which act as reservoir of virus for next season. Similar observation was given during field survey conducted in cucurbit crops in Pakistan [17]. Agricultural intensification also attributed to Begomovirus disease outbreaks [10,18-21].

3.2 Screening for Resistance through Graft Transmission under *in vitro* Condition

The purpose of this experiment was to identify ridge gourd with resistance against mosaic disease. Four days after grafting, new flushes

from rootstock had characteristic symptoms. Yellow mosaic, blisters and stunted growth were observed on majority of plants and detected for the presence of Begomovirus with an amplicon size of 575 bp approximately. After 20 days of grafting, the hybrid MHRG 7 recorded a high VI (65.33) and Arka Vikram recorded a low VI (39.33). After 40 days of grafting, hybrid Sorot F1 recorded high VI (78) and Arka Sujat recorded low VI (48). After 60 days of grafting, KAU released variety KRH-1 recorded the highest VI (91.33) while Arka Vikram recorded the lowest VI (66.67).

Based on the VI after 60 DAG, treatments were categorized into different genotypic classes [22]. Plants with 0 VI values are categorized under immune (I). Plants showing 1-25% VI are resistant (R). Plants with 26-50%, 51-75%, 76-100% VI categorized under moderately resistant (MR), moderately susceptible (MS) and susceptible (S) respectively. Present study revealed the moderately susceptible reaction of RG7 and RG10 ridge gourd plants. All other treatments are susceptible to the virus infection (Fig. 3).

Table 1. Disease incidence and vulnerability index of ridge gourd viruses in surveyed locations

Sl.No	Location	Disease incidence (%)	Vulnerability Index
1	Kunjathur	100	57.6
2	Thalangady	100	84.08
3	Kundukolake	76.47	39.44
4	Hosabettu	100	77.92
5	Karuvachery	17.85	42.64
6	Valiyaparamba	39.06	30.56
7	Thrikaripur	32.30	26.57
8	Azhikode north	26.56	40
9	Kalliasseri	5.3	49.5
10	Kolathuvayal	11.67	53.05
11	Kuttyeri	90	61.2
12	Thaliparamba	8.4	38.29
13	Mavichery	76.6	43.44
14	Cherukulathur	68	34.88
15	Chathamangalam	35	26.57
16	Poolacode	35	35.71
17	Avala	0	0
18	Akkuparamb	0	0
19	Velom	0	0

Table 2. Screening of varities/hybrids for resistance to ridge gourd mosaic virus

Code	Treatments	Source	VI 20 DAG	VI 40 DAG	VI 60 DAG
RG1	Haritham	Kerala Agricultural University (KAU)	55.33[bcd]	72.00[bc]	84.00[c]
RG2	Arka Sujat	IIHR, Bengaluru	45.33[ab]	48.00[a]	80.67[bc]
RG3	Arka Prasan	IIHR, Bengaluru	64.00[d]	76.67[bc]	88.00[c]
RG4	MHRG 7	Mahyco Private Limited	65.33[d]	72.67[bc]	82.67[bc]
RG5	KRH -1	KAU	64.00[d]	73.33[bc]	91.33[d]
RG6	Local 1	Local genotype	62.67[cd]	77.33[c]	86.00[c]
RG7	Local 2	Local genotype	48.00[abc]	68.67[bc]	70.00[ab]
RG8	Sorot F1	East- West Seed International	58.67[bcd]	78.00[c]	86.00[c]
RG9	NS 474	Namdhari Seeds Private Limited	54.00[abcd]	68.67[bc]	77.33[abc]
RG10	Arka Vikram	IIHR, Bengaluru	39.33[a]	62.00[ab]	66.67[a]
	SE (m)		5.30	5.16	4.61
	CD (0.05)		15.65	15.23	13.61

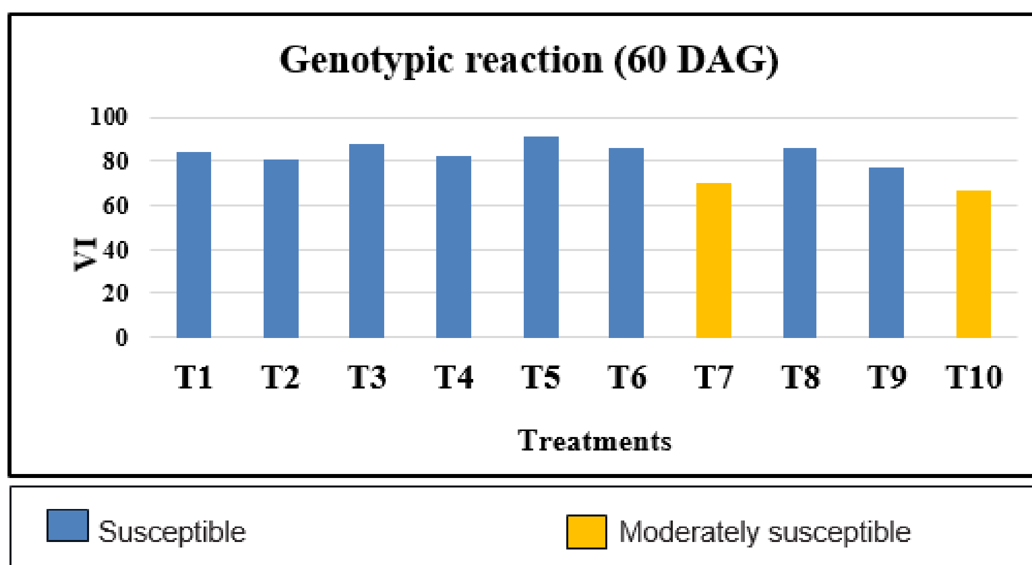

Fig. 3. Response of different varieties/hybrids of ridge gourd to virus disease upon grafting (60 DAG)

Grafting helps to screen resistant genotype in a very short time than conventional breeding procedures. By employing the right rootstocks, one can increase vigour, precocity, production, quality, survival rate, reduced infection by soil-borne pathogens, and increased resistance to abiotic stresses [23,24]. Resistance source for RgYMD was screened in seventy- six varieties/hybrids ridge gourd plants by insect transmission with *Bemisia tabaci*. None of them were found to be resistant, but highly susceptible [6]. In order to assess the degree of resistance to a viral disease, 53 distinct bitter gourd accessions were studied [25]. The analysis indicated that there was no genotype that showed complete resistance to the viral infection. Ridge gourd varieties 'Dodka', 'Janhi', 'Jhinga Tori', and 'Dundul' were found to be relatively tolerant to the *Ridge gourd mosaic virus* (RGMV) in field conditions [26]. *Lufa acutangula* genotype IIHR-Sel-1 and IIHR-137 is yellow mosaic resistant genotype and Arka Prasan is yellow mosaic susceptible genotype [27].

Begomoviruses (ToLCNDV) uses suppressor proteins (VSRs) encoded by AV2, AC2, and AC4 ORFs (pathogenicity factors) to avoid host RNAi mechanism which results in virus infection and susceptible reaction in susceptible plants [28].

3.3 Molecular Detection, Characterization and Phylogenetic Analysis of DNA

DNA isolated from samples collected from each two taluk of Kasaragod, Kannur and Kozhikodeand from weeds -*Synedrella* sp. and *Alternanthera* sp. were subjected to PCR for detection of DNA virus by using AV/AC primers. Amplicon size was 575 bp for each samplesdetected (Fig. 4,5).

The sequence from ridge gourd virus isolate exhibited 93 per cent nucleotide identity with ToLCNDV in Blast analysis [29]. *Luffa acutangula* virus (EU366163) isolate has 99–98% sequence similarity with three isolates of ToLCNDV (EF063145, EF123060, and EU439261) [5]. *Luffa cylindrica* virus (EU439261) shared 99–98% similarity with ToLCNDV – Bottle gourd (DQ272540), ToLCNDV – Tomato (EF123060), ToLCNDV – Cotton (EF063145), ToLCNDV – Potato (DQ272541) and ToLCNDV – Chilli (DQ141676). A studyrevealed 92.3 per cent nucleotide sequence similarity of DNA- A component of ridge gourd with ToLCNDV- [IN:Spo:05]. Similarly, DNA B component shared (83.1%) sequence identity with DNA-B of ToLCNDV-[IN:ND:Rid:10]strain [6].

Sequenced DNA was analyzed with NCBI-BLAST (National Center for Biotechnology Information-Basic Local Alignment Search Tool). On analysis, DNA from Thalangady (Kasaragod) showed 97.53% similarity with *Tomato leaf curl New Delhi virus* isolate TN MET Coc 1 coat protein (AV1) gene, complete cds (KM275605.1). DNA from Kolathuvayal (Kannur) indicated 98.88% nucleotide similarity with *Tomato leaf*

curl New Delhi virus isolate Bangalore 1 coat protein gene (MT708589.1). Mavichery (Kannur) isolate had 98.8 % similarity with *Tomato leaf curl New Delhi virus* isolate BG MIS GT coat protein gene with accession number OP096429.1. Thrikaripur isolate (Kasaragod) observed 98.83% similarity with *Tomato leaf curl New Delhi virus* isolate BG MIS GT coat protein gene (OP096429.1). Poolacode (Kozhikkode) isolate indicated 99.40 % similarity with *Tomato leaf curl New Delhi virus* isolate BG MIS GT.

Phylogenetic tree was constructed with MEGA 11 software by neighbour joining method and analyzed the relationship between each isolate of surveyed area. Thrikaripur, Mavichery and Poolacode isolates were closely related to each other (Fig. 7).

3.4 Isolation and Detection of Genomic RNA

RNA isolated by Trizol method from samples collected during survey and converted to cDNA. cDNA was further subjected to PCR by using specific primers of CMV -CMV1 and CMV2 primers. With an amplicon size of 400 bp, associated RNA virus in the surveyed location was detected (Fig. 6). Nucleic acid-based amplification using coat protein specificprimers to ToLCNDV CP gene was reported [30]. The CP is an essential component of *Begomovirus* survival and has been widely used to characterize and establish the relationships of many begomoviruses. Since it is a conserved area, it is sufficient for the initial detection and sequence analysis [31].

Evolution of Begomovirus strain in newer geographical areas might be due to recombination or mutation occuring in genes [32,33]. Evidence for intra specific recombination in DNA-A component was given by analysing the sequences derived from both ToLCNDV-[TW: Ori:07]-GU180095 (major parent) and ToLCNDV-[IN: Bah:Chi:07]-EU309045 (minor parent) collected from surveyed location [6]. It also facilitated by the vector population especially whitefly in epidemic regions. A study revealed that combined infections of distinct Begomoviruses in a common host plant could lead to recombination [34].

3.4.1 Molecular detection of RNA

Presence of *Cucumber mosaic virus* was detected using CMV1and CMV2 primers from ridge gourd samples collected during survey with an amplicon size of 400bp. Bottle gourd samples having mosaic mottling symptoms collected from Tamil Nadu undergone RTPCR and observed amplicon size of 1200 bp [35].

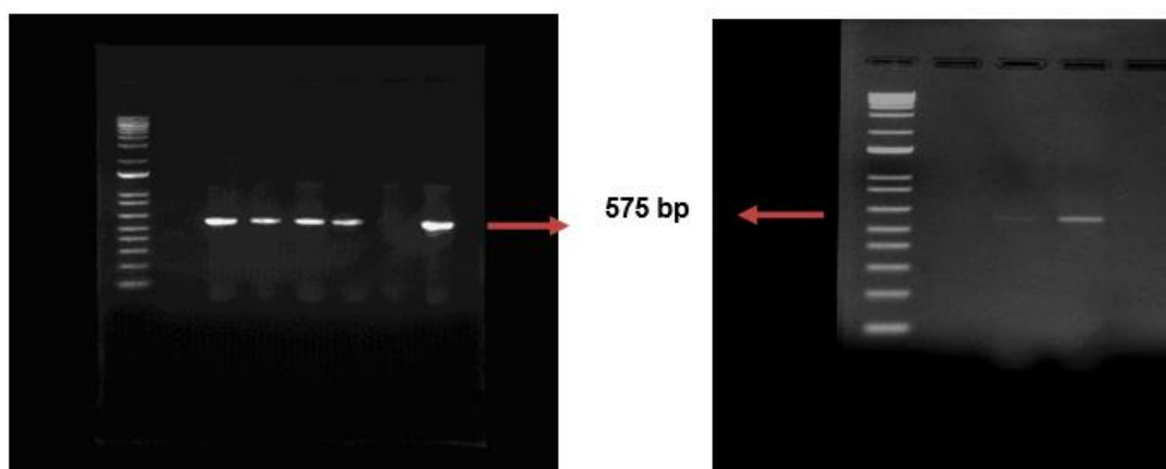

Fig. 4. Gel electrophoretic image of DNA extracted from ridge gourd of surveyed location with coat protein primer (AV/AC) of Begomovirus Lane 1- I kb ladder, Lane 2-Control, Lane 3-Kuttyeri isolate, Lane 4- Thrikaripur isolate, Lane 5- Thaliparamba isolate, Lane 6- Poolacode isolate, Lane 7-Avala isolate, Lane 8- Thalangady street isolate

Fig. 5. Gel electrophoretic image of DNA extracted from weeds collected during survey with coat protein primer (AV/AC) of Begomovirus- Lane 1- 1 kb Ladder, Lane2-Control, Lane3Alternanthera sp., Lane 4- *Synedrella sp.*

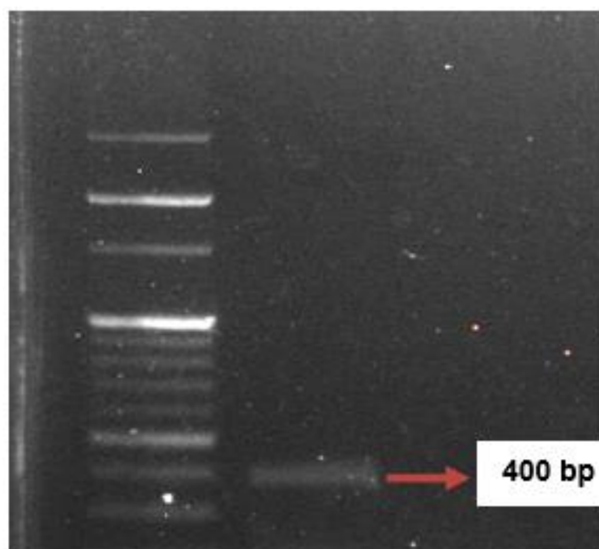

Fig. 6. Gel electrophoretic image of amplicon with coat protein specific CMV primers Lane 1-1kb ladder, Lane 2- Ridge gourd

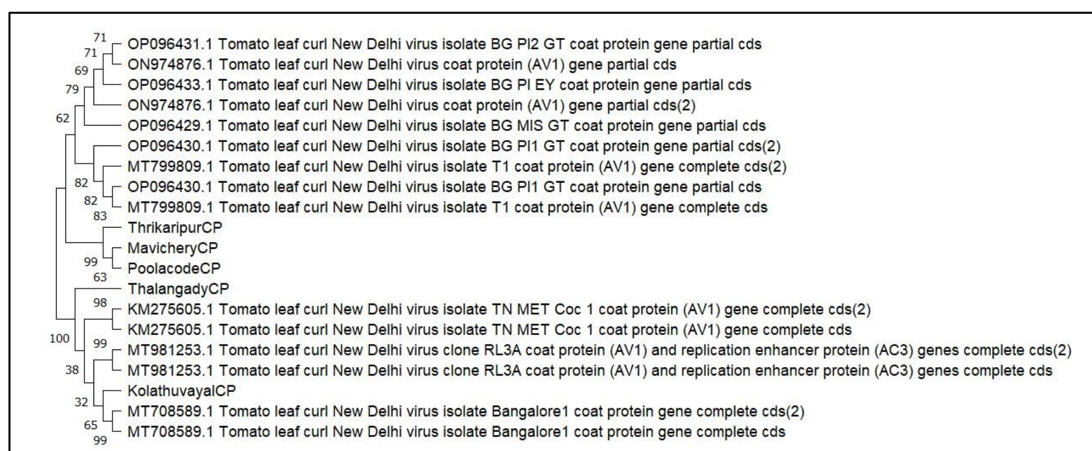

Fig. 7. Phylogenetic tree of Begomovirus constructed with isolates from different surveyed locations and already reported sequences in the NCBI database

4. CONCLUSION

The study revealed that the ridge gourd cultivated in areas of Kasaragod, Kannur and Kozhikode were infected with ToLCNDV and CMV; RG7 and RG10 were moderately susceptible among the varieties and hybrids screened. The presence of the Begomovirus was detected in weeds indicating its role in perpetuation and spread of the virus disease in the field. Future emphasis should be in the standardization of an early detection method for the multiple viruses involved and the development of an integrated management strategy for virus disease associated with ridge gourd.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAOSTAT [Food and Agriculture Organization]. Statistical database [on-line]. Available:http://www.fao.org/faostat/en [Accessed 09 November 2022]
2. Chakravarty HH. Cucurbits in India and their role in development of vegetable crops. In: Bates DM, Robinson RW, Jeffrey C. (eds.), Biology and Utilization of

the Cucurbitaceae. Cornell University Press, Ithaca, New York. 1990;325-348.

3. Kandoliya UK, Marviya GV, Bodar NP, Bhadja NV and Golakiya BA. Nutritional and antioxidant components of ridge gourd (*Luffa acutangula* L. Roxb) fruits of promising genotypes and varieties. Sch J Agric Vet Sci. 2016;3(5):397-401.
4. Lecoq H, Katis N. Control of cucurbit viruses. Adv Virus Res. 2014;90:255-296.
5. Tiwari AK, Snehi SK, Singh R, Raj SK, Rao GP, Sharma PK. Molecular identification and genetic diversity among six begomovirus isolates affecting cultivation of cucurbitaceous crops in Uttar Pradesh, India. Arch Phytopathol Plant Prot. 2012; 45(1):62-72.
6. Patil CV, Ramdas SV, Premchand U, Shankarappa KS. Survey, symptomatology, transmission, host range and characterization of begomovirus associated with yellow mosaic disease of ridge gourd in southern India. Virus Disease. 2017;28(2):146-155.
7. Nagendran K, Pandey KK, Rai AB, Singh B. Viruses of vegetable crops: Symptomatology, diagnostics and management. IIVR Technical Bulletin. 2017;75:1-60.
8. Kumari S, Krishnan N, Dubey V, Das B, Pandey KK, Singh J. Investigations on annual spreading of viruses infecting cucurbit crops in Uttar Pradesh state, India. Sci Rep. 2021;11(1):1-17.
9. Asad Z, Ashfaq M, Inam-Ul-Haq M, Irshad G, Khan MA. Current status and molecular characterization of zucchini yellow mosaic virus (ZYMV) infecting ridge gourd (*Luffa acutangula* L.) in different regions of Punjab, Pakistan. Pak J Bot. 2022;54(2): 467-474.
10. Varma A, Malathi VG. Emerging geminivirus problems. A serious threat to crop production. Ann Appl Biol. 2003; 142:145-164.
11. Brown JK, Zerbini FM, Castillo NJ, Moriones E, Sobrinho RR, Silva JC, Olive FE, Briddon, RW, Zepeda HC, Idris A, Malathi VG. Revision of begomovirus taxonomy based on pairwise sequence comparisons. Arch Virol. 2015;160:1593-1619.
    Available:https://doi.org/10.1007/s00705-015-2398-y
12. Sohrab SS. Variability in the geminiviruses infecting cucurbits. PhD thesis, Jamia Milia Islamia, New Delhi. 2005;58.
13. Wyatt SD, Brown JK. Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. Phytopathol. 1996;86:1288-1293.
14. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30:2725-2729.
15. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanater-phenol-chloroform extraction. Anal Biochem. 1987;162(1):156-159.
16. Bos L. Crop losses caused by viruses. Adv Virus Res. 1982;2:31-57.
17. Ali A, Hussain A, Ahmad M. Occurrence and molecular characterization of Cucumber green mottle mosaic virus in cucurbit crops of KPK, Pakistan. Braz J Microbiol. 2014;45:1247-1253.
18. Morales FJ, Anderson PK. The emergence and dissemination of whitefly-transmitted geminiviruses in Latin America. Arch Virol. 2001;146:415- 441.
19. Xie Y, Zhou XP. Molecular characterization of squash leaf curl Yunnan virus, a new begomovirus and evidence for recombination. Arch Virol. 2003;148:2047-2054.
20. Thresh JM. Cropping practices and virus spread. Annu Rev Phytopathol. 1982;20: 193–218.
21. Matson PA, Parton WJ, Power AG, Swift MJ. Agricultural intensification and ecosystem properties. Sci. 1997;277:504–509.
22. Havey MJ. CMV resistance in three sources of cucumber. Cucurbit Genet Coop. 1996;19:32-33.
23. Lee JM, Oda M. Grafting of herbaceous vegetables and ornamental crops. Hort Revi. 2003;28:61-124.
24. Buller S, Inglis D, Miles C. Plant growth, fruit yield and quality, and tolerance to verticillium wilt of grafted watermelon and tomato in field production in the Pacific Northwest Hortic Sci. 2013;48(8):1003-1009.
25. Asna AC, Joseph J, Kurien PS, John KJ. Identification of bitter gourd genotypes with field tolerance to viral diseases. J Trop Agric. 2018;56(1).

26. Singh RP, Mohan J and Singh DP. Assessment of losses and management of Ridgegourd mosaic virus. Plant Dis Res. 1999;14:134-138.
27. Kaur M, Varalakshmi B, Mahesha B. Mechanical sap transmission of Tomato leaf curl New Delhi virus infecting ridge gourd [*Luffa acutangula* (L.) Roxb.] in south India. Int J Chem Stud. 2020;8(3): 1867-1870.
28. Moriones E, Praaven S, Chakraborty S. Tomato leaf curl new delhi virus: An emerging virus complex threatening vegetable and fiber crops. Viruses. 2017;9: 264.
29. Manjunath HS, Rangaswamy KT, Basavaraj S, Bhagyashree M, Nagaraju N, Prameela HA. Molecular detection and identification of tomato leaf curl new delhi virus associated with yellow mosaic disease of ridge gourd (*luffa acutangula* l.) Based on coat protein gene. Int J Agric Sci. 2016;61(8):3444-3449.
30. Sohrab SS, Mandal B, Ali A, Varma A. Chlorotic curly stunt: A severe Begomovirus disease of bottle gourd in Northern India. Indian J Virol. 2010;21:56–63.
31. Fauquet CM, Stanley J. Geminivirus classification and nomenclature: Progress and problems. Ann Appl Biol. 2003; 142(2):165-189.
32. Harrison BD, Swanson MM, Fargette D. Begomovirus coat protein: Serology, variation and function. Physiol Mol Plant Pathol. 2002;60:257–271.
33. Kirthi N, Maiya SP, Murthy MRN, Savitri HS. Evidence of recombination among the Tomato leaf curl virus strains/species from Bangalore, India Arch Virol. 2002;147:255–272.
34. Maruthi MN, Rekha AR, Muniyappa V. Pumpkin yellow vein mosaic disease is caused by two distinct begomoviruses: Complete viral sequences and comparative transmission by an indigenous *Bemisia tabaci* and the introduced B-biotype. Bull. 2007;37:412–419.
35. Nagendran K, Priyanka R, Aravintharaj R, Balaji CG, Prashant S, Basavaraj B, Mohankumar S, Karthikeyan G. Characterization of *Cucumber mosaic virus* infecting snake gourd and bottle gourd in India. Physiol Mol Plant Pathol. 2018;103: 102-106.

© 2024 Sana et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/111624