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ORIGINAL ARTICLE

# Survey, symptomatology, transmission, host range and characterization of begomovirus associated with yellow mosaic disease of ridge gourd in southern India

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**Abstract** Ridge gourd is an important vegetable crop and is affected by several biotic and abiotic factors. Among the different biotic factors, ridge gourd yellow mosaic disease (RgYMD) is new emerging threat for the production of ridge gourd. The incidence of the RgYMD varied from 30 to 100% in southern India with highest disease incidence of 100% observed in Belagavi district of Karnataka state. The infected plants showed chlorosis, mosaic, cupping of leaves, blistering, reduction in leaf size and stunted growth. The varieties/hybrids grown in the farmer's fields were found to be susceptible to the disease. Begomovirus was detected in 61 out 64 samples collected from different areas of southern India. Further, all the samples failed to give amplification for beta and alpha satellites. The transmission studies revealed that single whitefly (*Bemisia tabaci*) is enough to transmit the virus, however, 100% transmission was observed with 10 whiteflies. The minimum acquisition access period and inoculation access period for transmission of virus by whitefly was 15 min. Among the 56 host plants belonging to diversified families tested for host range, sponge gourd, ash gourd, bottle gourd, pumpkin, cucumber, summer squash, cluster bean, tobacco and datura were shown to be susceptible. Seventy six varieties/hybrids evaluated for identifying the resistance source for RgYMD, all were found highly susceptible. Sequence analysis of DNA-A revealed that the causal virus shared highest nucleotide sequence identity (92.3%) with Tomato leaf curl New Delhi virus (ToLCNDV) infecting sponge gourd from northern India. Sequence and phylogenetic analysis of both DNA-A and DNA-B components showed that the begomovirus associated with RgYMD is found to be strain of ToLCNDV. This is first report of ToLCNDV association with RgYMD from southern India.

**Keywords** Ridge gourd · Begomovirus · Yellow mosaic disease · Disease incidence · Polymerase chain reaction · Phylogenetic tree

## Introduction

Ridge gourd [*Luffa acutangula* (L.) Roxb.] belongs to Cucurbitaceae family and it is one of the important vegetable crops grown across the world. India is considered as a primary centre of origin for ridge gourd [10]. At present, it is being cultivated in India, Egypt, China, Japan, Southeast Asia and parts of Africa. The tender fruit is used as vegetable, which is easily digestible with high nutritive value [27]. Fiber is obtained from fully ripened and dried fruits which has multiple uses [11]. The quality and yield of this crop is getting reduced in recent days due to the pests and diseases. This crop is known to be affected by many diseases, among them, powdery mildew (*Sphaerotheca fulginea*), downy mildew (*Pseudoperonspora cubensis*), collar rot (*Rhizoctonia solani*) and Pythium rot (*Pythium butleri*) are important [13]. In India,

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begomoviruses have emerged as major constraint in cucurbits cultivation [44]. Recently, Ridge gourd yellow mosaic disease (RgYMD) caused by begomoviruses are becoming the major constraints for its production [2, 39, 43].

Ridge gourd yellow mosaic disease (RgYMD) has been shown to be associated with begomovirus from northern India [43] and Sri Lanka [2] based on coat protein (CP) gene sequence. In Saudi Arabia and Vietnam, RgYMD was found to be associated with Tomato yellow leaf curl virus (ToYLCV) [39] and Luffa yellow mosaic virus (LYMV) (AF509739), respectively. However, so far no complete genome information of begomovirus associated with RgYMD in India is available.

The emergence of several plant viruses in crop species poses a threat to food security and economic progress in different parts of the world [42]. The begomoviruses belongs to family *Geminiviridae*. Members of the family *Geminiviridae* have small single-stranded circular DNA with distinctive twinned isometric particle of 20 nm × 30 nm sized. The begomoviruses have either monopartite genome having single-stranded circular DNA (ss DNA) component of about 2.7 kb, or bipartite genomes having two similar size components designated as DNA-A and DNA-B [17, 33]. The component of DNA-A contains five open reading frames (ORFs) encoding the factors required for virus replication, overcoming host defenses, insect transmission and control of gene expression. DNA-B component contains two ORFs, encode factors required for inter and intra-cellular movement in host plants [32]. Mixed infection of begomoviruses resulting in recombination and pseudo-recombination among diverse species and thus lead to frequent emergence of novel begomoviruses [3, 15, 29, 34, 36, 46].

Satellite DNA molecules known as DNA β or betasatellites, have been found associated with monopartite begomoviruses and they depend upon their helper viruses for encapsidation, replication, insect transmission and movement in plant [6, 12, 16, 18, 35, 47]. Alphasatellites are self replicating circular ssDNA molecules and are evolved from nanoviruses, and they depend on the helper virus for movement, encapsidation and vector transmission and play no role in symptom induction [4, 5, 7, 21]. Further, the Rep proteins encoded by some alphasatellites posses suppressor activity of RNA silencing, which revealed that alphasatellites are associated in overcoming host defenses [28].

Currently, there is no information of begomoviruses infecting ridge gourd from southern India. With this backdrop, the present study on the symptomatology, incidence, detection, virus–vector relationships, host range and characterization of ToLCNDV causing RgYMD in southern India was taken up.

## Materials and methods

### Disease incidence

The roving surveys were carried out during March 2015 to April 2015 at several locations in different districts of Karnataka (Belagavi, Bijapur, Bagalkot, Kolar, Chikkaballapur, Bangalore rural, Ramanagara, Mandya, Mysore, Davangere, Shivamoga, Tumkur), Andhra Pradesh (Chittur and Ananthapur) and Tamil Nadu (Krishnagiri) to determine the prevalence of RgYMD. Disease incidence was assessed by counting number of symptomatic plants (symptoms viz. chlorosis, mosaic, cupping of leaves, blistering, reduction in leaf size and stunted growth) over total number of plants (including both symptomatic and non-symptomatic) in randomly selected plots (25 m long live fence or in 5 m × 5 m) at each location. During the survey, symptomatic leaf samples were collected from each location and designated as separate isolate for further studies.

### Detection of begomovirus

The total DNA was extracted from RgYMD affected ridge gourd leaf samples collected, healthy ridge gourd leaf samples from glasshouse and chilli leaf curl diseased sample (to serve as positive control for DNA-beta and alphasatellites) by CTAB method [19, 23]. To confirm the presence of begomovirus in ridge gourd samples, the PCR was employed to amplify CP gene as described by Sohrab et al. [40] with primers specific to ToLCNDV CP gene. These primers used based on reports of ToLCNDV associated with other gourds [1, 40]. Additional PCR reactions were carried to amplify full-length DNA-beta [5] and alphasatellite molecules [9].

### Maintenance of vector and virus cultures

The pure culture of whitefly (*B. tabaci*) used for the current investigation was originally collected from brinjal (*Solanum melongena*) at experimental plots, KRC College of Horticulture, Arabhavi, Gokak taluk, Belagavi district, Karnataka, India. Culture was maintained on cotton (*Gossypium hirsutum* cv. Varalakshmi) in insect proof wooden cages.

Virus isolate associated with RgYMD showing typical symptoms was collected from naturally infected ridge gourd plants in the farmer's field of Sanganakeri village, Gokak taluk, Belagavi district, Karnataka, India during March 2014 and was maintained in the glasshouse for further studies. The disease was established on healthy seedlings (eight days old) of ridge gourd ($F_1$ hybrid; Naga)

under controlled condition through whitefly (*B. tabaci*) transmission with an AAP and IAP of 24 h each. The virus culture was maintained in ridge gourd plant inside a insect proof net house by regularly inoculating with viruliferous whiteflies.

### Transmission studies

To test the effect of number of whiteflies on virus transmission, one, two, three, five, 10, 15 and 20 viruliferous whiteflies per plant was used. 20 ridge gourd plants were used for each transmission experiment. The non-viruliferous *B. tabaci* were given an AAP of 24 h on infected ridge gourd plant and released on healthy ridge gourd plant separately, with the numbers mentioned above and were allowed for 24 h of IAP. Later, whiteflies were killed by spraying with 0.05% imidacloprid 17.8SL and plants were kept in an insect-proof glasshouse for symptom expression to record per cent transmission in each case.

The effect of different AAP on the rate of virus transmission was tested by allowing *B. tabaci* to feed for five, 10, 15, 30 min and one, three, six, 12 h and 24 h on RgYMD affected ridge gourd plants separately. After the prescribed AAP, the whiteflies were transferred on to eight days old healthy ridge gourd seedlings at the rate of 15 whiteflies per plant and 24 h as IAP was given. The plants were kept in the glasshouse for symptoms development.

To determine the influence of different IAP on transmission of virus, *B. tabaci* were allowed for 24 h as AAP on RgYMD plant. Viruliferous whiteflies were then transferred to eight days old healthy ridge gourd seedlings at the rate of 15 whiteflies per plant, separately and allowed IAP for five, 10, 15, 30 min and one, three, six, 12 and 24 h. Whiteflies were then killed by insecticide spraying as described earlier and seedlings were kept in an insect-proof net house for symptom development.

### Host range

In order to determine the host range, the virus was inoculated through *B. tabaci* to 56 plant species belonging to different families i.e. Amaryllidaceae, Amarantheceae, Apiaceae, Asteraceae, Brassicaceae, Caricaceae, Chenopodiaceae, Cucurbitaceae, Euphorbiaceae, Leguminosae, Malvaceae, Moringaceae, Plantagonaceae Solanaceae and Umbelliferae. Adult whiteflies were allowed to feed on symptomatic leaves for 24 h as AAP, there after 30 whiteflies were transfer to individual test seedling for 24 h as IAP. Inoculated plants were maintained for symptom expression for 60 days post inoculation (dpi) in insect proof net house.

### Screening ridge gourd hybrids and varieties for resistance to ridge gourd yellow mosaic disease

Seventy six ridge gourd cultivars/varieties/hybrids obtained from different sources were screened for resistance to RgYMD under glass house conditions. Eight days old seedlings were inoculated with virus using 30 viruliferous *B. tabaci* that were given 24 h as AAP and IAP.

### Characterization, cloning and sequencing

Full length genomes were amplified using the overlapping primers specifically designed to amplify DNA-A and DNA-B components of begomoviruses [45]. PCR products were electrophoresed (1 h at 80 v) on 1% agarose gel and viewed on a gel documentation system (Alpha Innotech, USA). Amplified PCR products were purified from gel using MinElute gel extraction kit (Qiagen, Germany) and separately ligated in pTZ57R/T vector using InsTAclone PCR cloning kit (Thermo Fisher Scientific, USA), according to the manufactures instructions. The plasmid DNA was isolated from positive clones by using alkaline lysis method and sequenced in both directions using universal M13 forward and reverse primers at Chromus Biotech, Pvt Ltd, Bangalore, India.

### Sequence analysis

The sequences of DNA-A and DNA-B components of the present isolate were compared with the sequences of begomoviruses retrieved from the GenBank (Supplementary Tables 1 and 2). To find the location of each gene/ORFs, analysis was carried out using the online software 'ORF finder' at http://www.ncbi.nlm.nih.gov/gorf/gorf.html. The nucleotide sequences of DNA-A, DNA-B and amino acid (aa) sequences of the ORFs present in the genomes were analyzed using CLUSTALW multiple alignment tool to obtain the percent identities. Phylogenetic trees were generated using MEGA software (version 7.0) by following Maximum Parsimony method with 60% cut off value and 1000 bootstrap replicates [41].

### Recombination analysis

The sequence of DNA-A of selected begomoviruses obtained from GenBank (NCBI) (Supplementary Table 1) along with the isolate characterized in the present study were used in recombination analysis using the Recombination detection program (RDP), GENECOV, Bootscan, Max Chi, Chimara, Si Scan, 3Seq which are integrated in RDP4 to detect the recombination break points [22]. Default RDP settings with 0.05 $p$ value cut-off throughout and standard Bonferroni correction were used.

**Table 1** Distribution and incidence of yellow mosaic disease of ridge gourd in Karnataka, Andhra Pradesh and Tamil Nadu states, India and confirmation of presence of begomoviruses by PCR

| State | District | Disease incidence | | Varieties/hybrids grown | PCR detection |
|---|---|---|---|---|---|
| | | Range | Average | | No of sample found positive in PCR/No of sample tested |
| Karnataka | Bagalkot | 65–100 | 82.6 | Anamika, NS-3, Sunitha, Pallavi, Saniya, Mahyco-7, Naga, Karod local, Mahyco-3, Jamakandi local, Mahyco-1 | 17/18 |
| | Bangalore rural | 40–50 | 45.0 | UGR 113, Naga, NS-3 | 3/3 |
| | Belagavi | 72–100 | 88.0 | Jaipur long, Naga, Nagin | 4/4 |
| | Bijapur | 75–88 | 82.0 | Mayuri, Pradham Padmini, Mahyco-1, Anamika, Jaipur long, Mahyco-3, NS-3, Naga, Sarika-9, Saniya | 8/10 |
| | Chikkaballapura | 30–40 | 35.0 | Mahyco-7, Mayuri, Pradham Padmini, Anamika | 4/4 |
| | Davanagere | 35–90 | 65.8 | Naga | 3/3 |
| | Kolar | 35–50 | 38.0 | NS-3, Mahyco-3, Pallavi, Saniya | 4/4 |
| | Mandya | 30–55 | 43.3 | Mahyco-3, Naga, Jaipur long | 3/3 |
| | Mysore | 30–50 | 41.6 | Naga | 3/3 |
| | Ramanagara | 45–50 | 47.5 | Naga, Sarika | 2/2 |
| | Shivamoga | 35–82 | 55.8 | Naga, NS-3 | 5/5 |
| | Tumkur | 35–40 | 37.5 | Naga, BSS-582 | 2/2 |
| Andhra Pradesh | Ananthapur | 35 | 35 | Saniya | 1/1 |
| | Chittur | 50 | 50 | Naga | 1/1 |
| Tamil Nadu | Krishnagiri | 30 | 30 | Saniya | 1/1 |

## Results

### Disease Incidence, symptoms and detection

RgYMD was prevalent in all the surveyed areas. Disease incidence ranged from 30 to 100%. In Karnataka, minimum disease incidence (35%) was recorded at Chikkaballapur district, where as maximum (100%) at Belagavi district (Table 1). In Andhra Pradesh, minimum disease incidence (35%) was recorded in Ananthapur district and maximum (50%) in Chittur district. In Tamil Nadu, 30% disease incidence was observed at Krishnagiri district. The survey data revealed that all the varieties/hybrids (Naga, NS-3, Jaipur long, Sarika, UGR 113, Anamika, Pradham, BSS-582, Mayuri, Saniya, Pallavi, Mahyco-3, Mahyco-1, Mahyco-7, Sarika-9, Sunitha, Nagin, Karod local, Jamakandi local) grown were susceptible to RgYMD. Amongst them, the maximum infection (100%) was observed in $F_1$ hybrid *i.e.,* Naga (Table 1).

Naturally infected ridge gourd plants showed typical mosaic with light chlorotic areas on the leaf lamina, yellow, pale green intermingled with normal green tissues, reduction in leaf size, upward curling, blistering of leaves and stunting of plant (Fig. 1a–d).

PCR was employed to samples surveyed to amplifying CP gene of ToLCNDV using primers specific to coat protein gene [40]. Of the 64 samples tested, 61 showed desired amplification (Table 1), which indicates the occurrence of RgYMD associated with ToLCNDV in the three states of southern India. No amplification was found in DNA extracted from the healthy plants confirming that the PCR products were virus specific. PCR also was employed for detecting the beta and alpha-DNA satellites from the samples. The successful amplification in positive control and no amplification in the ridge gourd samples

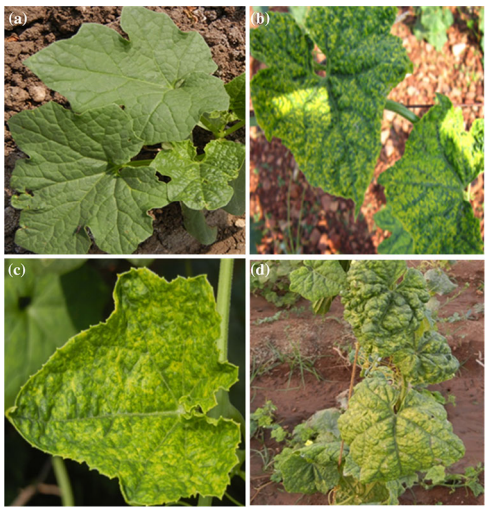


**Fig. 1** Symptoms of ridge gourd yellow mosaic disease under field conditions, **a** light chlorotic specks, **b** yellow, pale green intermingled with normal green tissues, **c** cupping of leaf and **d** reduction of growth

**Table 2** Determination of acquisition access periods (AAP) and inoculation access periods (IAP) of Begomovirus associated with Ridge gourd yellow mosaic disease using *Bemisia tabaci*

| AAP | IAP (h) | Average transmission efficiency[a] | IAP | AAP (h) | Average transmission efficiency[a] |
|---|---|---|---|---|---|
| 5 min | 24 | 0 | 5 min | 24 | 0 |
| 10 min | 24 | 0 | 10 min | 24 | 0 |
| 15 min | 24 | 20 | 15 min | 24 | 15 |
| 30 min | 24 | 40 | 30 min | 24 | 25 |
| 1 h | 24 | 60 | 1 h | 24 | 40 |
| 3 h | 24 | 70 | 3 h | 24 | 80 |
| 6 h | 24 | 90 | 6 h | 24 | 90 |
| 12 h | 24 | 100 | 12 h | 24 | 100 |
| 24 h | 24 | 100 | 24 h | 24 | 100 |

[a] No of plant showing symptom over 20 no of plants inoculation in each case

collected in the current study indicated that, the absence of satellite molecules in those samples.

## Transmission

The transmission of casual virus using whitefly showed 100% transmission in ridge gourd. The ridge gourd plants developed characteristic symptoms within 8–12 dpi. Studies on virus-vector relationship revealed that single adult whitefly was able to transmit the virus with 20%, while 10 adult whiteflies per plant gave 100% transmission of the virus. The transmission rate increased with an increase in number of whiteflies per plant. Whitefly required minimum AAP and IAP of 15 min each (Table 2). Hundred percent transmission of the virus was achieved with AAP and IAP of 12 h using 15 whiteflies (Table 2).

## Host range

Of the 56 plant species used in host range studies, six plant species [a) pumpkin, b) summer squash, c) bottle gourd, d) sponge, e) cucumber (*Cucumis sativus* L.), f) ash gourd] belonging to Cucurbitaceae family, one plant species belongs to Leguminosae i.e. g) cluster bean and three plant species [h) tobacco (*Nicotiana tabacum* L (Domin), i) tobacco *(N. benthamiana*) and j) datura (*Datura stromanium* L.)] belonging to Solanaceae (Fig. 2a–j) (Table 3) found susceptible to RgYMD. Sponge gourd has showed 100% infection with vein clearing, yellow mosaic following by curling, cupping and mottling of leaves. More than 50% infection was observed in pumpkin, cucumber, cluster bean, datura and tobacco. Lowest infection was observed in ash gourd (10%) summer squash (35%) and bottle gourd (40%) (Table 3).

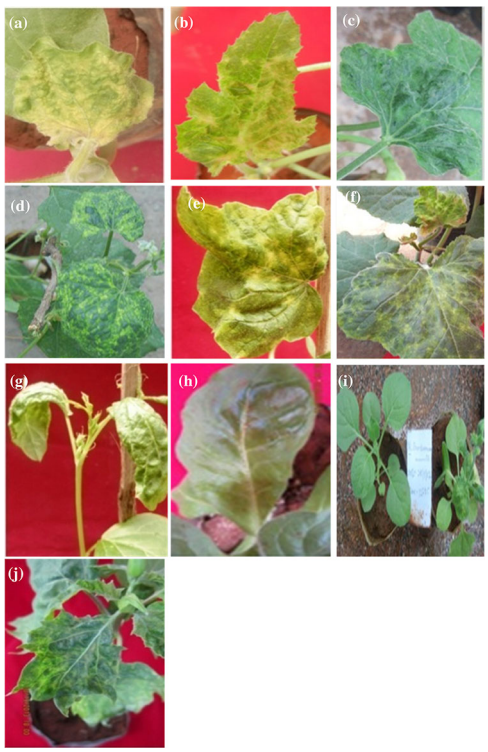


**Fig. 2** Symptoms on different plant species after whitefly inoculation of ToLCNDV-[INDIA:Karnataka:Ridge gourd:2014], **a** pumpkin, **b** summer squash, **c** bottle gourd, **d** sponge gourd, **e** cucumber, **f** ash gourd, **g** cluster bean, **h** tobacco (*Nicotiana tabacum* L (Domin) **i** tobacco (N. *benthamiana*) and **j** datura

## Screening of hybrids and varieties of ridge gourd against yellow mosaic disease

All the 76 varieties/hybrids evaluated against RgYMD exhibited highly susceptible reaction with 100 percent disease incidence (Supplementary Table 3).

**Table 3** Host range of yellow mosaic disease of ridge gourd assessed by symptoms expression after virus inoculation by adult *Bemisia tabaci* in glass house conditions

| Sl. no. | Plant species | Common name | Variety | Family | No. of Infected/ inoculated[a] |
|---|---|---|---|---|---|
| 1 | *Luffa cylindrica* L. Roem. | Sponge gourd | KRCCH-1 | Cucurbitaceae | 20/20 (100) |
| 2 | *Cyamopsis tetragonaloba* L (Taub.) | Cluster bean | Pusa Navabahar | Leguminaceae | 28/35 (80.0) |
| 3 | *Cucurbita moschata* L. (Duch) ex I am.) Duch I ex Pon. | Pumpkin | SPH-201 | Cucurbitaceae | 25/33 (75.7) |
| 4 | *Datura stromanium* L. | Datura | – | Solanaceae | 18/25 (72.0) |
| 5 | *Cucumis sativus* L. | Cucumber | Green long | Cucurbitaceae | 19/30 (63.3) |
| 6 | *Nicotiana benthamiana* Domin | Tobacco | – | Solanaceae | 12/20 (60.0) |
| 7 | *Nicotiana tabacum* L *(Domin)* | Tobacco | – | Solanaceae | 11/20 (55.0) |
| 8 | *Legenaria segeraria* (Molina.) Standl. | Bottle gourd | KRCCH-1 | Cucurbitaceae | 8/20 (40.0) |
| 9 | *Cucurbita pepo* L. | Summer squash | Selection KGP | Cucurbitaceae | 7/20 (35.0) |
| 10 | *Benincasa hispida* (Thumb) Cogn. mosaic. | Ash gourd | KRCCH-1 | Cucurbitaceae | 2/20 (10.0) |
| 11 | *Allium sativum* L. | Garlic | Yamuna Safed (G-1) | Amaryllidaceae | 0/30 (0) |
| 12 | *Allium cepa* L. | Onion | Arka Nikethan | Amaryllidaceae | 0/30 (0) |
| 13 | *Amaranthus tricolor* L. | Amaranthus | Gokak local | Amarantheceae | 0/30 (0) |
| 14 | *Coriandrum sativum* | Coriander | Arabhavi local | *Apiaceae* | 0/30 (0) |
| 15 | *Callistephus chinensis* L. | China aster | Kamini | Asteraceae | 0/30 (0) |
| 16 | *Dendranthema grandiflora* L. | Chrysanthemum | Dundi | Asteraceae | 0/30 (0) |
| 17 | *Parthenium hysterophorus* | Parthenium | Local | Asteraceae | 0/30 (0) |
| 18 | *Tagetes erecta* L. | Marigold | Local | Asteraceae | 0/20 (0) |
| 19 | *Helianthus annuus* | Sunflower | Sunflower-44 | Asteraceae | 0/30 (0) |
| 20 | *Brassica oleracea* var. *capitata* | Cabbage | Harnil | Brassicaceae | 0/30 (0) |
| 21 | *Brassica oleracea* var. *botrytis* | Cauliflower | Pusa Meghana | Brassicaceae | 0/22 (0) |
| 22 | *Raphanus sativus* L. | Radish | Gokak local | Brassicaceae | 0/30 (0) |
| 23 | *Carica papaya* L. | Papaya | Agro seeds | Caricaceae | 0/30 (0) |
| 24 | *Beta vulgaris* | Palak | Arka Anupama | Chenopodiaceae | 0/30 (0) |
| 25 | *Beta vulgaris* L. | Beetroot | Gokak local | Chenopodaceae | 0/25 (0) |
| 26 | *Cucumis melo* L. | Musk melon | Hariha | Cucurbitaceae | 0/30 (0) |
| 27 | *Citrulus lanatus* L. | Water melon | NS-295 | Cucurbitaceae | 0/30 (0) |
| 28 | *Momordica charantia* L. | Bitter gourd | Arka Harit | Cucurbitaceae | 0/20 (0) |
| 29 | *Trichosanthus cucumerina* L | Snake Guard | Syndrella | Cucurbitaceae | 0/20 (0) |
| 30 | *Cucurbita maxima* Duch. Ex L am. | Bush Squash | Pratty Pan | Cucurbitaceae | 0/20 (0) |
| 31 | *Praecitrullus fistulosus* Pang. | Round Melon | Arka Tinda | Cucurbitaceae | 0/20 (0) |
| 32 | *Euphorbia geniculata* | Euphorbia weed | Local | Euphorbiaceae | 0/30 (0) |
| 33 | *Arachis hypogaea* | Ground nut | TMV-2 | Leguminosae | 0/30 (0) |
| 34 | *Trigonella foenum-graecum* | Kasurimetthi | Local | Leguminosae | 0/30 (0) |
| 35 | *Cicer arietinum* | Bengal gram | JG-11 | Leguminosae | 0/30 (0) |
| 36 | *Vigna unguiculata* | Cowpea | Local | Leguminosae | 0/30 (0) |
| 37 | *Phaseolus vulgaris* L. | French bean | Arka Komal | Leguminosae | 0/30 (0) |
| 38 | *Vigna radiata* L. *(Wilczek.)* | Green gram | Local | Leguminosae | 0/30 (0) |
| 39 | *Cajanus cajan* | Pigeon pea | Local | Leguminosae | 0/30 (0) |
| 40 | *Cassia angustifolia* | Senna | Local | Leguminosae | 0/30 (0) |
| 41 | *Dolichos lablab* L. | Dolichos bean | Sarpan Hybrid-3 | Leguminosae | 0/30 (0) |
| 42 | *Foenum graecum* L. | Methi | Gokak local | Leguminosae | 0/30 (0) |
| 43 | *Glycine max* L. | Soya bean | Gokak local | Leguminosae | 0/30 (0) |
| 44 | *Pisum sativum* L. | Pea | Agro seeds | Leguminosae | 0/30 (0) |
| 45 | *Gossypium hirsutum* | Cotton | Local | Malvaceae | 0/30 (0) |
| 46 | *Abelmoschus esculentus* | Okra | Arka Anamica | Malvaceae | 0/30 (0) |

**Table 3** continued

| Sl. no. | Plant species | Common name | Variety | Family | No. of Infected/ inoculated[a] |
|---|---|---|---|---|---|
| 47 | *Hybicus subdarifa* | Pundi | Local | Malvaceae | 0/30 (0) |
| 48 | *Abelmoschus moschata* | Kasturibhendi | Local | Malvaceae | 0/30 (0) |
| 49 | *Moringa oleifera* | Drumstick | Bhagya | Moringaceae | 0/30 (0) |
| 50 | *Pantago ovata* | Isabgol | Local | Plantagonaceae | 0/30 (0) |
| 51 | *Capsicum annum* L. | Chilli | Arkha Lohit | Solanaceae | 0/25 (0) |
| 52 | *Capsicum frutescence* L. | Capsicum | California Wonder | Solanaceae | 0/30 (0) |
| 53 | *Nicotiana glutinosa* L. *(Domin)* | Tobacco | – | Solanaceae | 0/15 (0) |
| 54 | *Solanum lycopersicon* L. *(Mill.)* | Tomato | Arkha Lohit | Solanaceae | 0/30 (0) |
| 55 | *Solanum melongena* L. | Brinjal | KRCCH-1 | Solanaceae | 0/30 (0) |
| 56 | *Daucus carota* | Carrot | Pusa Yamadagni | Umbelliferae | 0/30 (0) |

AAP = 24 h IAP = 24 h

[a] Thirty whiteflies per plant were used for inoculation, values in parentheses indicate the percent disease incidence

## Characterization

The PCR amplification of full genome components of begomovirus associated with RgYMD was carried out using six pairs of overlapping primers. The PCR products were cloned and sequenced. The sequences of these amplicons were aligned and the overlapping sequences were removed to get a complete sequence of DNA-A. DNA-A component consisted of 2739 nucleotides which contain seven genes. Further, the nucleotide and amino acid sequence percent identities of RgYMD isolate with other begomoviruses sequences showed maximum identities with ToLCNDV infecting different crop plants (Supplementary Table 4). On comparison, the nucleotide sequence of full length DNA-A component of begomovirus isolated from ridge gourd with other begomovirus isolates revealed that the begomovirus under study showed highest nucleotide sequence identity (92.3%) with ToLCNDV-[IN:Spo:05] (Supplementary Table 4). The isolate under present study shares more than 91% sequence identity with other ToLCNDV isolates so, it is considered as another isolate. Based on its sampling location, host and year of collection, the isolate under present study has been given a descriptor as tomato leaf curl New Delhi virus–[INDIA:Karnataka:Ridge gourd:2014] with its abbreviation as ToLCNDV-[IN:Kar:Rid:14]. Phylogenetic tree based on alignment of complete DNA-A nucleotide sequence of the begomovirus isolate under study with that of other selected begomoviruses revealed that the begomovirus infecting the ridge gourd clustered with ToLCNDV isolates and formed a distinct branch within the cluster (Fig. 3a).

The PCR amplification of DNA-B genome of begomovirus infecting the ridge gourd using three sets of overlapping primers resulted in three genomic fragments. The amplified products were cloned and sequenced. The sequences of these three amplicons were aligned and the overlapping sequences were removed to obtain a complete sequence of DNA-B. A complete nucleotide sequence of DNA-B was determined to be 2694 nucleotides. The genome organization was similar to that of previously described bipartite begomoviruses. The nucleotide sequence identity ranged from 77.3 to 83.1% among the ToLCNDV isolates. On comparison, the nucleotide sequence of full-length DNA-B component of begomovirus isolated from ridge gourd with other begomovirus (Supplementary Table 5) isolates revealed that the isolate under study had highest nucleotide sequence identity (83.1%) with DNA-B of ToLCNDV-[IN:ND:Rid:10]. This was supported by phylogenetic analysis of DNA-B nucleotide sequence showing the isolate under study clustering with the ToLCNDV (Fig. 3b).

## Recombination analysis

The recombination analysis of DNA-A component of ToLCNDV-[IN:Kar:Rid:14] showed that the Gokak virus isolate contains the sequences derived from major parent ToLCNDV-[TW:Ori:07]-GU180095 and the minor parent ToLCNDV-[IN: Bah:Chi:07]-EU309045 with the break point starting at 692$^{nd}$ and ending at 1479$^{th}$ nucleotide position for all recombination detection methods applied with the *p* values of $4.412 \times 10^{-03}$ (RDP), $3.854 \times 10^{-02}$ (Bootscan), $2.946 \times 10^{-04}$ (Chimera), $2.321 \times 10^{-11}$ (SiScan) and $1.663 \times 10^{-01}$(Maxchi) (Supplementary Table 6). Results indicated that there is intra specific recombination.

## Discussion

Begomoviruses associated diseases are becoming major constraints for the production of cucurbits in the Indian sub-continent. The increased incidences of begomoviruses

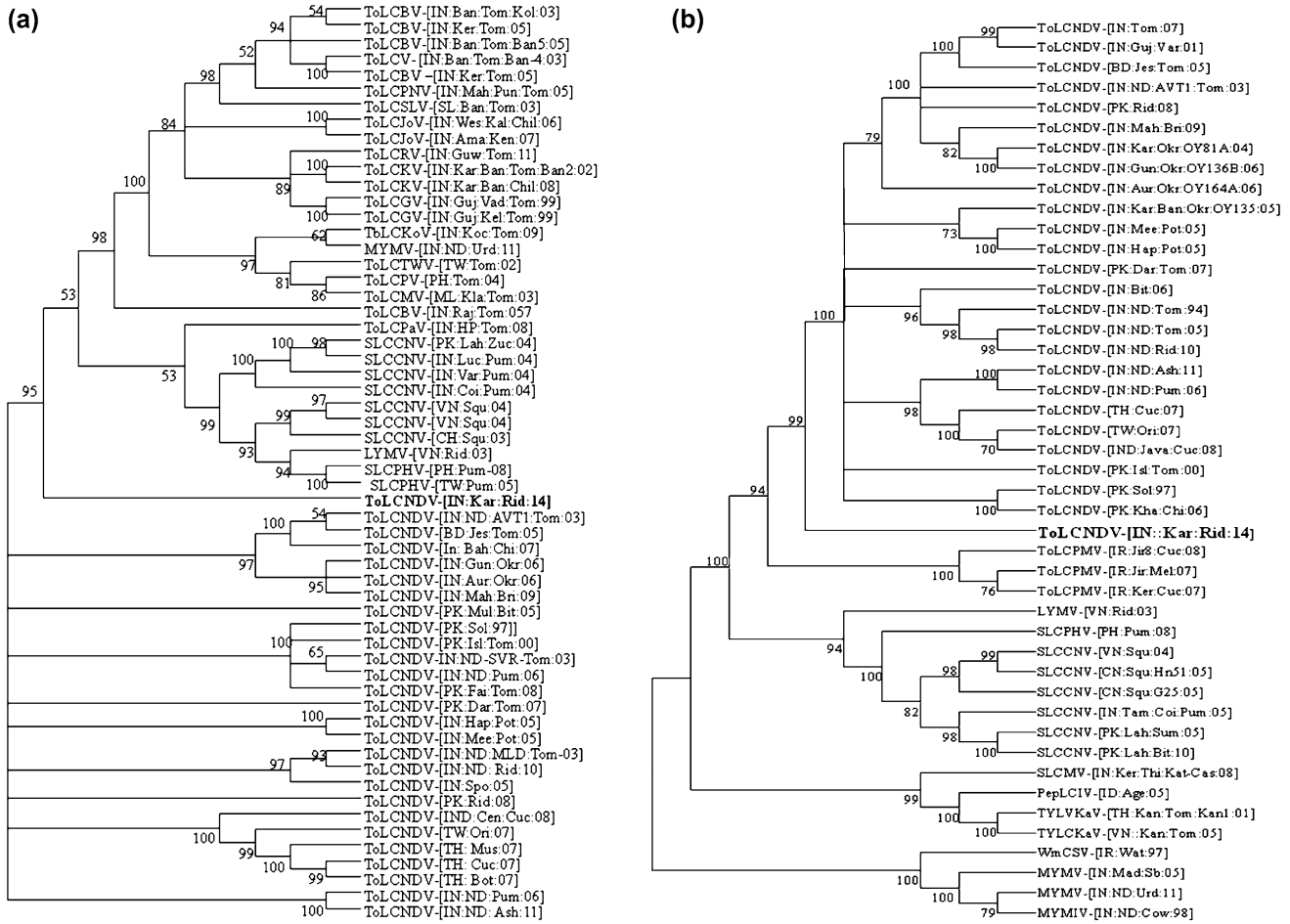


**Fig. 3** The phylogenetic tree showing the relationship of begomovirus isolated from ridge gourd with other selected begomoviruses based on their full length DNA-A (**a**) and DNA-B (**b**) nucleotide sequences. The number at each node indicates ≥60% bootstrap values with 1000 replicates

associated diseases in the recent time are attributed to the introduction of whitefly biotypes with more efficiency in transmission of virus and high fecundity and, reduction in the genetic diversity of the crop [37]. Ridge gourd is one of the important vegetable crops in India and it is suffering from several biotic and abiotic stresses [13]. The RgYMD is emerging problem in ridge gourd [2, 39, 43]. The results of survey revealed that RgYMD is prevailing in all the areas surveyed with varying percent incidence. Highest disease incidence observed at Belagavi district of Karnataka state, might be due to continuous availability of ridge gourd throughout the year with highly susceptible variety/hybrid. All the varieties/hybrids grown in the surveyed areas are found susceptible to disease and there is a need to identify resistance source against to RgYMD.

The studies on virus-vector relationship revealed that single whitefly was sufficient to transmit the virus. There was increase in the percent transmission with increase in number of whiteflies, AAP and IAP. Similar trend was reported in different begomoviruses like pumpkin yellow vein mosaic virus (PYVMV) [14, 24], crotan yellow vein mosaic virus (CYVMV) [20], Indian cassava mosaic virus (ICMV) [25], horsegram yellow mosaic virus (HYMV) [26] and tomato leaf curl virus (ToLCV) [31]. In the present investigation, for the first time a biological characterization of begomovirus associated with RgYMD from southern India was carried out.

The host range of the casual virus is limited to only few families of the test plants. However, six plant species in the Cucurbitaceae family tested were found susceptible indicating the possible extent of damage this virus can inflict on these crops. Further, whitefly could transmit the virus into tobacco, cluster bean and datura suggesting the other crop plants and weeds could serve as reservoir for the virus inoculum. Further knowing the all possible collateral hosts for the virus will help in the management of the disease.

For effective management of viral diseases, identification of resistant cultivars/genotypes is most important and the best practice to reduce yield loss. Susceptible reaction of all the varieties/hybrids screened for resistance to

RgYMD suggests that there is need to screen more number of germplasm against to disease and also avoid growing of these evaluated varieties/hybrids in disease prevailing areas.

The begomovirus isolated from ridge gourd had both DNA-A and DNA-B genomic components typical to the bipartite begomoviruses reported across the world infecting the different cucurbitaceous crops viz, ash gourd [1] and pumpkin [30]. Comparison of nucleotide identity and phylogenetic analysis of both genomic components clearly revealed that, the current isolate could be considered as a strain of ToLCNDV as per present ICTV begomovirus species demarcation [38]. This indicated the further expansion of host range of ToLCNDV to ridge gourd and becoming the potential threat for ridge gourd cultivation in southern India.

The contribution of ToLCNDV-[TW:Ori:07] and ToLCNDV-[IN: Bah:Chi:07] as major and the minor parent, respectively for emergence of DNA-A through the intraspecific recombination in further confirms the process of recombination and pseudorecombination result in evolution of begomoviruses and important source of variability of begomoviruses [38].

With this we conclude that, it is the first report from southern India providing valuable insights into the strain of ToLCNDV infecting ridge gourd causing RgYMD based on biological and molecular approaches.

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