

Overview of R&D of Plant Molecular Virology

1. Objectives:

- Biological, serological and molecular characterization of plant viruses and phytoplasmas affecting ornamental, horticultural and crop plants.
- Development of diagnostics for plant viruses and phytoplasmas for their sensitive detection in economically important crops.
- Development of virus-free and virus resistant transgenic plants of commercial importance.

2. Goals:

- Molecular identification and characterization of plant viruses affecting economically important crops and understanding the molecular variability exists among them for their proper taxonomic status worldwide.
- Development of diagnostics of plant viruses for their sensitive detection in ornamental, horticultural and crop plants.
- Development of virus-free and virus resistant transgenic plants for improvement of quality and production of economically important crops.

3. Competencies :

Plant molecular virology group has expertise in biological and molecular characterization of plant viruses; development of virus diagnostics; generation of virus-free plants and virus resistant transgenic plants. The group has characterized various newly emerging strains/isolates/species of Begomoviruses, Cucumoviruses, Potyviruses, Nepoviruses, Babuviruses, Nanoviruses/Satellite molecules and phytoplasma infecting various economically important crop plants and developed their management strategies.

4. Facilities:

All the basic and molecular facilities for detection and study the molecular biology of DNA and RNA viruses are available such as: PCR machine, Table top refrigerated centrifuge, Gel documentation system, -80⁰C and -20⁰C refrigerators, Multi tem Water bath, ELISA plate reader, Incubator shaker, Laminar flow, Hybridization oven, Tissue culture facility, glass house facility.

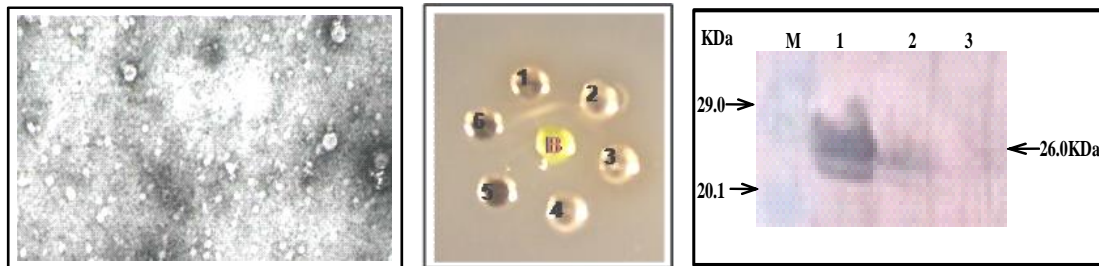
5. **Highlights of Current Research:**

a. **Biological, serological and molecular characterization of *Cucumber mosaic virus* infecting economically important crops plants:**

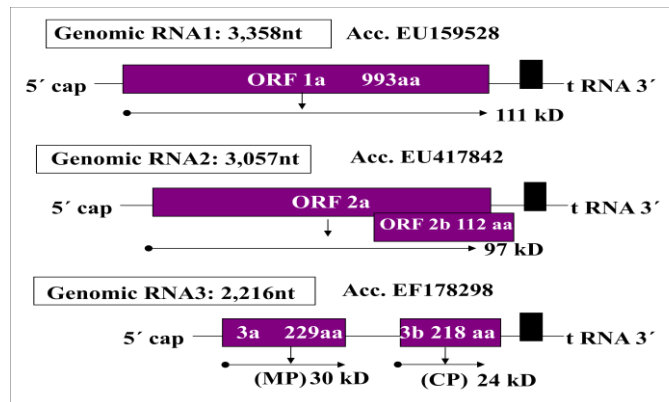
Biological and molecular characterization and genetic diversity of six strains of *Cucumber mosaic virus* infecting Amaranth, Datura, Caster bean, Chrysanthemum, Tomato, Banana and Gerbera plants.



Cucumber mosaic virus infected plants showing various symptoms such as: mosaic on banana, Datura, Chrysanthemum; blisters on tobacco, castor bean, Amaranths and Shoestring on tomato.



Immuno-sorbent electron microscopical assay, Gel double diffusion tests and Western blot immuno-assay diagnostics developed for *Cucumber mosaic virus* in Banana, Tomato, Gladiolus and Chrysanthemums.



Tripartite genome organization of *Cucumber mosaic virus* infecting banana and tomato investigated.

b. Molecular identification of potyviruses from ornamentals:

Three potyvirus strains viz. Tuberose mild mosaic on Tuberose (*Polianthes tuberosa*), Amyrallis mosaic virus on Amaryllis and *Ornithogalum mosaic virus* potyviruses on gladiolus were investigated. Recently, *Bean yellow mosaic virus* infecting Gladiolus, *Vicia faba*, Canna, Gooseberry and has been detected and characterized based on sequence analyses of polyprotein gene. The 9.0 kb genome of BYMV infecting Gladiolus has also been cloned and sequenced (Accession Num. KM114059).



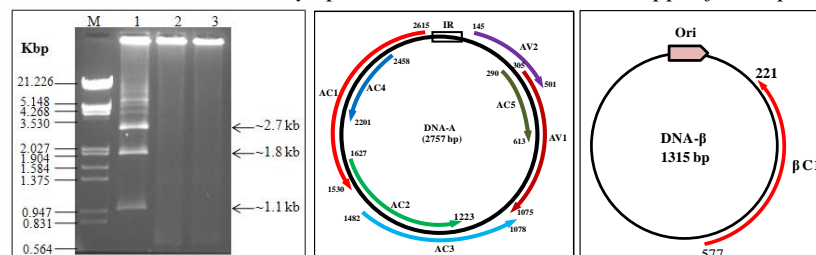
BYMV infected Gladiolus, Vicia faba and Gooseberry showing symptoms and a flexuous rod shaped virus particle observed in Electron microscope.

c. Molecular characterization of begomovirus infecting important crops plants:

Begomoviruses such as *Squash leaf curl China virus* of Pumpkin; *Tomato leaf curl New Delhi virus* of chili; *Croton yellow vein mosaic virus* and *Jatropha yellow mosaic India virus* of *J. gossypifolia*; *Jatropha mosaic India virus* of *J. curcas* and *Ageratum enation virus* of amaranths and poppy have been identified and characterized on the basis of sequence analyses of 2.7 kb cloned DNA-A genome amplified by rolling circle amplification using Phi29 DNA polymerase followed by restriction digestion by *Bam*HI.



Yellow mosaic and mosaic symptoms on J. curcas and other spp. of Jatropha

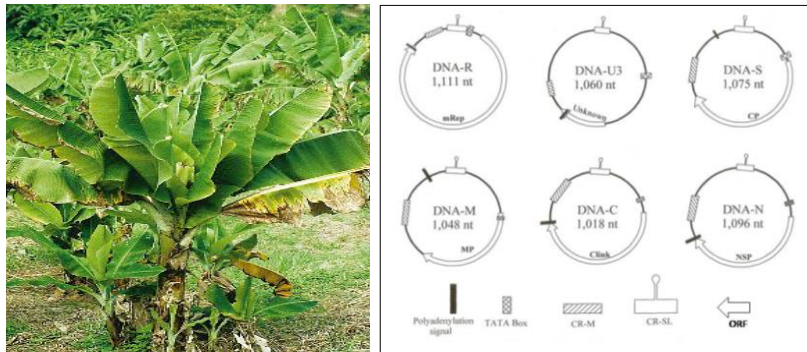


RCA amplification of DNA-A component of begomovirus infecting Jatropha. Monopartite genome of 2.7Kb DNA-A and 1.3 kb DNA-beta investigated with mosaic disease of Jatropha gossypifolia.

The agroinfectious clones of DNA-A genome of *Ageratum enation virus* causing yellow net and enation disease in amaranths and poppy have been generated and infiltrated to confirm the infectivity of the DNA molecule. The associations of betasatellite and alphasatellite molecules have also been investigated as symptom modulator of the begomovirus disease complexes in amaranth, poppy and ornamental *Ageratum* species.

d. Molecular characterization of Banana bunchy top virus infecting Banana:

Banana bunchy top virus (BBTV) is a single stranded circular DNA virus of the genus Babuvirus, belonging to family Nanoviridae. The six genomic DNA components of Indian (Lucknow) isolate of BBTV were amplified by PCR with specific primers using total DNA extracted from banana tissues showing typical symptoms of banana bunchy top disease (BBTD). The resulting ~1.1 Kb amplicons were cloned and sequenced. Analysis of sequence data revealed the presence of six full-length components of BBTV: DNA-R, DNA-U3, DNA-S, DNA-M, DNA-C and DNA-N. Comparisons of sequence data of the six DNA components of the BBTV Lucknow isolate revealed highest identities with sequences of other BBTV isolates from the South Pacific group. The phylogenetic analysis revealed a close relationship of the Lucknow isolate with BBTV isolates of South Pacific group rather than those of the Asian group. Therefore, the virus has been classified as BBTV Lucknow, a new member of South Pacific group.



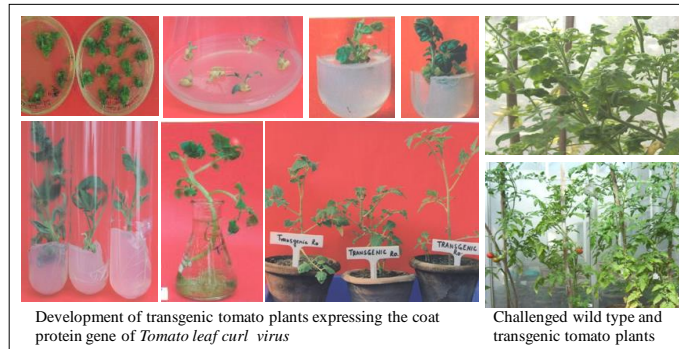
Bunchy top symptoms on banana and six genomic DNA components of Indian strain of Banana bunchy top virus causing bunchy top disease in Banana.

e. Molecular detection and identification of four groups of phytoplasma from economically important plants:

‘*Candidatus Phytoplasma asteris*’ (16SrI group) on Rose, Desert rose, Marigold, Gladiolus, Hibiscus, Chrysanthemum, chili and Gerbera; ‘*Candidatus Phytoplasma trifolii*’ (16SrVI group) with little leaf disease of *Datura innoxia* and Brinjal; ‘*Candidatus Phytoplasma cynodontis*’ (16SrXIV) on Bermuda grass and ‘*Candidatus Phytoplasma ziziphi*’ (16SrV group) on jujube (*Ziziphus* spp.).

f. Agrobacterium-mediated tomato transformation and regeneration of transgenic lines expressing *Tomato leaf curl virus* coat protein gene:

Pusa Ruby tomato leaf explants were transformed by co-cultivation with *Agrobacterium* containing TLCV–CP construct. Kanamycin-resistant transformants were regenerated and established in glasshouse. T₀-generation putative transgenic plants obtained were screened by PCR, Southern and Northern hybridization tests and Western blot assay, which confirmed the incorporation and expression of the CP gene. CP expressing transgenic plants were self-pollinated. T₁-generation transgenic plants were challenged by TLCV through whiteflies which showed variable degrees of disease resistance/tolerance compared to the untransformed control.



g. Development of transgenic tomato plants expressing coat protein gene of an Indian isolate of *Cucumber mosaic virus*:

Transgenic tomato plants containing coat protein (CP) gene of *Cucumber mosaic virus* (CMV) subgroup IB were developed through *Agrobacterium* mediated transformation. The progenies of transgenic plants showed positive transgene, its expression and translation of 26 KDa protein, when evaluated by PCR, southern hybridization, northern hybridization and western blot immune-assay, respectively. The T₁ and T₂ generation plants were evaluated for resistance against challenge inoculations of CMV. Visual observations of challenged transgenic plants categorized them into complete resistant, tolerant and susceptible as compared to untransformed control plants.

h. Genetic transformation and development of *Cucumber mosaic virus* resistant transgenic plants of *Chrysanthemum morifolium* cv. Kundan:

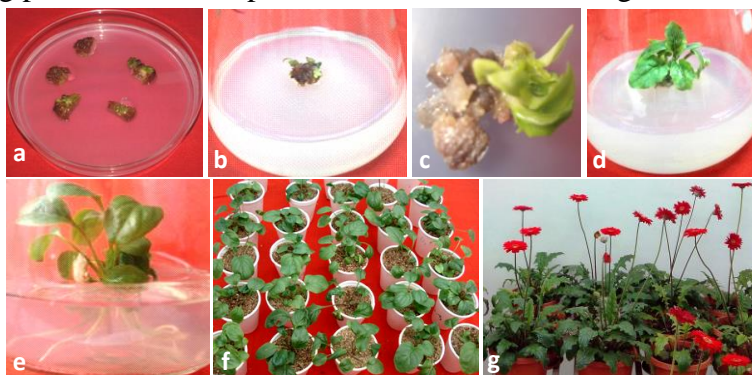
Agrobacterium-mediated transformation of petiole explants of *C. morifolium* was attempted using pRoK2 binary vector harbouring coat protein (CP) gene of CMV under the control of CaMV 35S promoter. A total of 257 explants were transformed and 73 putative transgenic plants from seven independent co-cultivation events were obtained with ~6% transformation efficiency. Molecular analysis of these plants confirmed the successful integration of CP transgene in 63% plants, of which 12.3% plants were able to transcript and translate the transgene. Expression of coat protein did not evoke any abnormal phenotype. Transgenic plants showed delayed resistance when challenged by CMV-chrysanthemum strain which produced good quality blooms.

- i. Elimination of mixed infection of *Cucumber mosaic* and *Tomato aspermy virus* from *Chrysanthemum morifolium* Ramat. cv. Pooja by shoot meristem culture:** Elimination of CMV and TAV was achieved by culturing 0.3mm long shoot meristem of infected plants on MS medium. The regenerated plants were indexed by DAC-ELISA and confirmed by RT-PCR. A total of 78.1% CMV and TAV-free shootlets were obtained from the regenerated shoot meristem as indexed by DAC-ELISA, of which only 65.6% were found truly virus-free when confirmed by RT-PCR. Virus-free shootlets were rooted on half MS medium and acclimatized under glasshouse. These plants showed better growth and quality of blooms as compared to diseased ones.



- j. Elimination of *Cucumber mosaic virus* from Zingaro cultivar of gerbera (*Gerbera jamesonii*) by *in vitro* chemotherapy for its quality improvement**

Natural infection of *Cucumber mosaic virus* (CMV) was detected in a number of gerbera (*Gerbera jamesonii*) cultivar Zingaro plants by RT-PCR using CMV-coat protein gene specific primers. The diseased plants exhibited severe yellow mosaic and flower distortion symptoms leading to deterioration of their market values. Elimination of CMV was attempted by *in vitro* chemotherapy (using 30 mg/l virazole) of ~4x8 mm² capitulum explants of infected gerbera for its quality improvement. A total of 38 plants were developed from 57 explants on MS medium supplemented with cytokinin and auxin hormones. The developed plants when screened by RT-PCR showed absence of CMV in 81.6% (31/38) plants. The CMV-free plants showed better plant growth and better blooming performance compared to the infected ones in glasshouse conditions.



Development of virus-free plants from infected floral bud explants of gerbera cv. Zingaro.

6.0 List of Ongoing projects:

A. Projects being handled at present (In House) OLP 0088

Genome organization, genetic variability and management of important plant viruses and Phytoplasmas affecting ornamentals, horticultural and other economically important crops - CSIR in house project (2007– continuing).

B. Projects being handled at present (BSC 0117) (CSIR Project)

Plant Microbe and Soil interaction: “Interaction of Potyvirus on Gladiolus and Cucumovirus on Gerbera”- (2012- continuing).

C. Project funded by Department of Science and Technology (GAP3317)

Molecular identification of Potyviruses infecting bulbous ornamental plants (2013- continuing)

7. Significant achievements:

- Molecular characterization of *Cucumber mosaic virus* strains infecting Amaranths, Datura, Chrysanthemum, tomato, banana, Petunia and Gerbera plants.
- Molecular identification of *Tomato aspermy virus* infecting Chrysanthemums and gladiolus.
- Molecular identification of *Banana bunchy top virus* affecting banana based on six DNA components.
- Complete genome characterization of two new begomovirus species: Jatropha yellow mosaic India virus from *J. gossypifolia* and Jatropha mosaic India virus from *J. curcas*.
- Complete genome characterization of *Ageratum enation virus* infecting amaranths, poppy and ornamental ageratum and associated betasatellite and alphasatellite molecules and their role in symptom modulation and disease development by Agro-infiltration assays.
- Detection of Tuberose mild mosaic from tuberose (*Polianthes tuberosa* L.), Amyrallis mosaic virus from Amyrallis and *Ornithogalum mosaic* potyvirus and *Bean yellow mosaic potyvirus* from gladiolus.
- Detection and identification of Phytoplasma isolates infecting Rose, Desert rose, Marigold, Gladiolus, Hibiscus, Chrysanthemum, Gerbera, chili, Sesame, Datura, Brinjal and jujube.
- Development of transgenic tomato lines expressing coat protein gene of *Tomato leaf curl virus* for resistance against TLCV.
- Development of transgenic tomato and chrysanthemum lines expressing coat protein gene of *Cucumber mosaic virus* resistance against CMV.
- Elimination of *Cucumber mosaic virus* and development of virus-free plants of chrysanthemum and Gerbera by *invitro* chemotherapy of their infected explants.

8. Last Five years Research Publications: (2009-2014)

38. A. Srivastava, M. Jaidi, S. Kumar and S. K. Raj (2014). Molecular identification of a new begomovirus associated with leaf crumple disease of *Jatropha curcas* L. in India. **Archives of Virology**. DOI: 10.1007/s00705-014-2288-8.

37. A. Srivastava, S. Kumar, S. K. Raj (2014). Molecular characterization and infectivity of a begomovirus and betasatellite associated with yellow vein net disease of *Ageratum houstonianum*. **Plant Disease**. dx.doi.org/10.1094/PDIS-03-14-0333-RE.
36. A. Srivastava, M. Jaidi, S. Kumar, S. K. Raj, S. Shukla (2014). First report of *Papaya leaf curl virus* association with leaf curl disease of grain amaranth (*Amaranthus cruentus* L.) from India. **Phytoparasitica** DOI: 10.1007/s12600-014-0424-3.
35. A. Srivastava, S. Kumar, S. K. Raj, S. S. Pande (2014) Association of a distinct strain of Hollyhock yellow vein mosaic virus and Ludwigia leaf distortion betasatellite with yellow vein mosaic disease of hollyhock (*Alcea rosea*) in India. **Archives of Virology** 159: 2711-2715.
34. K.K. Gautam, R. Raj, S. Kumar, S.K. Raj, R.K. Roy, R. Katiyar (2014). Complete sequence of RNA3 of *Cucumber mosaic virus* isolates infecting *Gerbera jamesonii* suggests its grouping under IB subgroup. **Virus Disease** 25: 398-401.
33. M.S. Khan, A. K. Tiwari, S. K. Raj, A. Srivastava, S. H. Ji and S. C. Chun (2014). Molecular epidemiology of begomoviruses occurring on some vegetables, grain legume and weed species in the Terai belt of north India. **Journal of Plant Diseases and Protection** 121: 53-57.
32. A. Srivastava, S. Kumar, S. K. Raj (2014). First report of *Ageratum enation virus*, betasatellite and alphasatellite causing leaf curl and enation disease of *Amaranthus hypochondriacus* in India. **Plant Disease** 98: 1285.
31. Charanjeet Kaur, Rashmi Raj, Susheel Kumar and S. K. Raj (2014). First report of *Bean yellow mosaic virus* on Cape gooseberry in India. **New Disease Report** 29: 17.
30. Arati Kumari, Susheel Kumar and Shri Krishana Raj (2014). First report of Canna yellow mottle virus on canna from India. **New Disease Reports** 29: 9.
29. A. Srivastava, S. Kumar, S. K. Raj (2014). Association of *Pedilanthus leaf curl virus* with yellow mottling and leaf curl symptoms in two jasmine species grown in India. **Journal of Genral Plant Pathology** 80: 370-373.
28. S. Kumar, K. K. Gautam, S. K. Raj (2014). Molecular identification of *Cucumber mosaic virus* isolates of subgroup IB associated with mosaic disease of eggplant in India. **Virus Disease** 25:129-131.
27. A. Srivastava, S. K. Raj, S. Kumar, S. K. Snehi (2013). New record of *Papaya leaf curl virus* and *Ageratum leaf curl betasatellite* associated with yellow vein disease of aster in India. **New Disease Reports** 28: 6.
26. A. Srivastava, S. K. Raj, S. Kumar, S. K. Snehi, A. Kulshreshtha, V. Hallan, S. S. Pande (2013). Molecular identification of *Ageratum enation virus*, betasatellite and alphasatellite molecules isolated from yellow vein diseased *Amaranthus cruentus* in India. **Virus Genes** 47:584-90.
25. R. Vishnoi, S. Kumar, S. K. Raj (2013). Molecular characterization of a *Cucumber mosaic virus* isolate associated with mosaic disease of banana in India. **Phytoparasitica**. 41: 545–555.
24. A. Srivastava, S. K. Snehi and S. K. Raj (2013) First report of a variant of *Pepper leaf curl Lahore virus* on winter cherry (*Solanum capsicastrum* L.) in India, **New Disease Reports** 27: 7.
23. C. Kaur, S. Kumar, S. K. Raj (2013) New record of association of *Bean yellow mosaic virus* with mosaic disease of *Vicia faba* in India. **Indian Journal Virology** 24: 95-96.

22. C. Kaur, S. Kumar, S. K. Snehi and S. K. Raj (2013) Molecular detection of Jasmine potyvirus associated with yellow mosaic symptoms on *Jasminum sambac* L. in India. **Archives Phytopathology and Plant Protection** 46:1102-1107.
21. J. A. T. da Silva, H. Shinoyama, R. Aida, Y. Matsushita, S. K. Raj and F. Chen (2013). *Chrysanthemum* Biotechnology: Quo vadis (Latin phrase meaning "Where are you going)?, **Critical Reviews in Plant Sciences** 32: 21-52.
20. K. K. Gautam, S. Kumar, S. K. Raj (2012). Molecular characterization of *Cucumber mosaic virus* strain causing severe mosaic disease on petunia in India. **Phytoparasitica**. 40: 425-431.
19. S. K. Snehi, A. Srivastava, S. K. Raj (2012). Biological Characterization and Complete Genome Sequence of a Possible Strain of Indian cassava mosaic virus from *Jatropha curcas* in India. **Journal of Phytopathology** 160:547-553
18. S. Srivastava, Hema Bisht, O. P. Sidhu, A. Srivastava, Poonam C. Singh, R M Pandey, S. K. Raj, Raja Roy, C. S. Nautiyal. (2012) Changes in the metabolome and histopathology of *Amaranthus hypochondriacus* L. in response to Ageratum enation virus infection. **Phytochemistry** 80: 8-16.
17. D. Pratap, S. K. Raj, S. Kumar, S. K. Snehi, K. K. Gautam, A. K. Sharma (2012). Coat protein-mediated transgenic resistance in tomato against a IB subgroup *Cucumber mosaic virus* strain. **Phytoparasitica** 40: 375-382.
16. D. Pratap, S. Kumar, S.K. Snehi and S.K. Raj (2012). Biological and molecular characterization of Cucumber mosaic virus isolate causing shoestring disease of tomato in India which has closer affinity to European or East Asian isolates of CMV. **Indian Journal Virology** 23: 57-63.
15. S. Kumar, S. K. Raj, A. K. Sharma and H. N. Varma (2012). Genetic transformation and development of *Cucumber mosaic virus* resistant transgenic plants of *Chrysanthemum morifolium* cv. Kundan, **Scientia Horticulturae** 134: 40-45.
14. A. Kumar, S. Kumar, S. K. Raj, J. Kumar and J. A. Khan (2011). Association of *Cotton leaf curl Burewala virus* and its satellite molecules with leaf distortion symptoms of cotton in India. **New Disease Reports** 24, 18.
13. S. K. Snehi, S. K. Raj, M. S. Khan and V. Prasad (2011). Molecular identification of a new Begomovirus species associated with yellow mosaic disease of *Jatropha gossypifolia* in India. **Archives of Virology** 156: 2303-2307.
12. C. Kaur, S. K. Raj, S. K. Snehi, A. K. Goel and R. K. Roy (2011). First report of natural occurrence of *Ornithogalum mosaic virus* on Gladiolus in India. **New Disease Reports** 24: 2.
11. S. K. Raj, S. K. Snehi, M. S. Khan, and A. K. Tiwari and G. P. Rao (2011). Molecular identification of *Ageratum enation virus* associated with mosaic disease of pointed gourd (*Trichosanthes dioica* Roxb.) in India, **Phytoparasitica** 39: 497-502.
10. S. K. Snehi, M. S. Khan, S. K. Raj and V. Prasad (2011). Complete nucleotide sequence of Croton yellow vein mosaic virus and DNA- β associated with yellow vein mosaic disease of *Jatropha gossypifolia* in India. **Virus Genes** 43: 93-101.
9. S. K. Raj, S. Kumar, D. K. Verma and S. K. Snehi (2011). First report on molecular detection and identification of *Tomato aspermy virus* naturally occurring on Gladiolus in India. **Phytoparasitica** 39: 303-307.

8. S. K. Raj, S. K. Snehi, K. K. Gautam and M. S. Khan (2010). First report of association of *Cucumber mosaic virus* with blister and leaf distortion of Castor bean (*Ricinus communis* L.) in India. **Phytoparasitica** 38: 283-289.
7. O. P. Sidhu, S. Annarao, U. Pathre, S. K. Snehi, S. K. Raj, R. Roy, R. Tuli and C. L. Khetrapal (2010). Metabolic and histopathological alterations of *Jatropha mosaic begomovirus* infected *Jatropha curcas* L. by HR-MAS NMR spectroscopy and Magnetic Resonance Imaging. **Planta** 232: 85-93.
6. S. K. Raj, S. K. Snehi, S. Kumar, T. Ram and A. K. Goel (2009). First report of *Tuberose mild mosaic potyvirus* on tuberose (*Polianthes tuberosa* L.) in India. **Australasian Plant Disease Notes** 4: 93-95.
5. S. K. Raj, S. K. Snehi, S. Kumar, B. K. Banerji, A. K. Dwivedi, R. K. Roy and A. K. Goel (2009). First report of 'Ca. P. asteris' (16SrI) associated with colour-breaking and malformation of floral spike of Gladiolus in India. **Plant Pathology** 58: 1170.
4. S. K. Raj, S. K. Snehi, S. Kumar and M. S. Khan (2009). First molecular detection and identification of a Potyvirus associated with severe mosaic disease of amaryllis (*Hippeastrum hybridum* Hort.) in India. **Australasian Plant Disease Notes** 4: 50-53.
3. R. Vishnoi, S. K. Raj and V. Prasad (2009). Molecular characterization of an Indian isolate of Banana bunchy top virus based on six genomic DNA components. **Virus Genes** 38: 334-344.
2. S. Kumar, M. S. Khan, S. K. Raj and A. K. Sharma (2009). Elimination of mixed infection of *Cucumber mosaic* and *Tomato aspermy virus* from *Chrysanthemum morifolium* Ramat. cv. Pooja by shoot meristem culture. **Scientia Horticulturae** 119: 108-112.
1. S. K. Raj, S. Kumar, S. Choudhari and D. K. Verma (2009). Biological and molecular characterization of three isolates of *Tomato aspermy virus* infecting chrysanthemums in India, **Journal of Phytopathology** 157: 117-125.

8. Scientists: (Name and Designation of Scientists working in the Research Area/R& D Groups).

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9. Technical Staff: (Name and Designation of Technical / Lab Astts./TOs in the Research Area/R& D Groups).

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Mr. M. J. Ansari (Lab Assistant)

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