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Decline of Mandarin Orange Caused by *Citrus tristeza virus* in Northeast India: Conventional and Biotechnological Management Approaches

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Abstract—*Citrus tristeza virus (CTV) is a phloem limited, aphid transmitted (Toxoptera citricida), longest known plant virus with 12×2000 nm particle, a member of genus Closterovirus (Family: Closteroviridae) and causes the severe decline (tristeza) and death of millions of citrus trees in the world. CTV infects all the commercial citrus species covering all the geographical zones in India. CTV has linear ssRNA genome of 20.3 kb with 12 ORFs. Major symptoms are quick decline, stem pitting, seedling yellows, vein clearing, vein flecking and vein corking. Based on extensive survey and indexing, the CTV incidence; 26.3% in Central (Vidarbha), 36-50% in South (Andhra Pradesh and Karnataka) and 16-60% in North & Northwest (Uttarakhand, Delhi and Punjab) and 47.1-56.0% in Northeast India (Assam, Meghalaya, Sikkim and the Darjeeling hills); was estimated. A total of 114 CTV isolates (16-19 of from central, 52-54 from Northeast, 17-18 from South and 14-15 North India) were characterized based on 5'ORF1a and coat protein (CP) gene. Indian isolates are extensive diverse showing 78-99% nt identity and fell into 7-9 different CTV genogroups. Intra-farm diversity of CTV in individual citrus farm is common in India.*

A decline inducing CTV strain Kpg3 in the mandarin orchards of the Darjeeling hills of India was identified. Complete genome of Kpg3 (19253nt; HM573451) was sequenced for the first time in India. The Kpg3 is a recombinant and genetically related to Israel severe CTV isolate VT. Based on the 3′ half genome (8.4 kb, ORFs 2-11), four CTV isolates; B5 (Bangalore: HQ912023), D1 (Delhi: HQ912022), G28 (Assam: KJ914661) and Kat1 (Vidarbha: KJ914662); were characterized and they have 89-99% nt identities among them. Genomes of Indian, Asian and International isolates were analysed and compared. The Asian isolates fell into six, whereas the Indian isolates into four genogroups. Indian isolates D1, Kat 1 and Kpg3 grouped together (Kpg3Gr). The B5 isolate of Bangalore is a new and the G28 of Assam is distinct isolate isolates/strains. The recombination phenomenon is the major factor for evolution of diversified CTV in India. Codon biasness, negative selection and gene flow also play major role for evolution of CTV variants.

Citrus bud wood and shoot tip grafting for production of disease free planting materials are being practiced in many citrus growing areas in India. CTV free mandarin planting materials was developed and supplied to the farmers of the Darjeeling hills. For development of transgenic resistant citrus plant, several antisence (RNAi) and hairpin (ihpRNAi) gene constructs targeting CP (p25) and suppressor (p23) gene of CTV in pBinAR and pCAMBIA2301 binary vector were made. Agrobacterium-mediated transformation protocol was developed using epicotyl explants of citrus seedlings. Regeneration efficiency of transformed plant was 1.38% at 2.0 mg/l BAP in MS medium. Mild cross protecting strains (MCPS) were identified by in silico codon usage biasness analysis of CP and p23 gene and evaluated through biological indexing challenging with severe CTV isolates.

1. INTRODUCTION

Citrus tristeza virus (CTV), an aphid-transmitted closterovirus, causes devastating 'Tristeza' or decline in most of the economically grown citrus species in the world. It causes phenomenal economic damage to citrus production globally destroying about 100 million citrus trees over the last 70 years (Moreno et al., 2008). As citrus is cultivated in diverse ecological conditions, it is exposed to several CTV strains resulting in diverse disease syndromes like decline of citrus species grafted on sour orange, yellowing and growth cessation of many citrus species and stunting and stem pitting with poor yield and quality in citrus species regardless of kind of citrus rootstock used.

CTV, a phloem-limited, flexuous filamentous virus with particle size of 2000 X 11 nm belongs to the genus *Closterovirus* under the family *Closteroviridae*. It is predominantly transmitted by brown citrus aphid (BrCA; *Toxoptera citricidus*) in a semi-persistent manner (Bar-Joseph and Lee, 1989). CTV genome is positive sense, ssRNA of 19.3kb in length and contains 12 ORFs; ORF1a and b and ORFs 2-11 potentially encoding at least 19 putative proteins (Karasev *et al*., 1995). CTV contains two capsid proteins: a major CP (CP) of 25 KDa (p25) and a minor CP (CPm) of 27 KDa (p27). ORFs 1a and b encode replication related proteins those are translated from genomic RNA (gRNA), whereas the other ten 3′ proximal ORFs encode different proteins p33, p6, p65, p61, p27 (CPm), p25 (CP), p18, p13, p20 and p23, those are expressed via 3′ co-terminal sub genomic RNAs (sgRNA) (Satyanarayana, 2000).

Genetic diversity in CTV in different citrus growing regions of the world has been reported earlier. Analysis with several

complete genomes of CTV isolates shows extensive sequence variation in CTV genome and determines at least seven CTV genotypes; T36, VT, T30, T3, B165, HA16- 5 and RB (resistance breaking) occurring in citrus growing countries in the world (Melzer et al., 2010; Biswas et al., 2012a). Of them, genotypes VT is biologically severe, T30 as mild and and T36 as intermediate strains of CTV (Anonymous, 2012). CTV infect all the citrus species, and citrus relatives and hybrids and a non-rutaceous host *Passiflora* sp (Bar-Joseph and Dawson, 2008). This virus is transmitted by aphids in a semi-persistent manner.

2. INCIDENCE OF CTV INDIA

Citrus is cultivated in all the four geographical zones of India; Northeast, Northwest, Central and South Northeast (NE) India is considered as one of the most important centres of origin of different citrus species (Ghosh, 2007). CTV is a major problem to cause decline of citrus orchards in NE India (Ahlawat 1997, Bhagabati *et al*, 1989). Several mandarin orchards in the Darjeeling hills are reported to be affected by CTV severely causing huge economic losses and many of them are being wiped out (Ahlawat, 1997; Biswas, 2008). Assam, and Meghalaya produce important citrus fruits like Khasi mandarin (*C. reliculata*), Acid lime/Kagzilime and Assam lemon (*C. lemon*) and CTV is reported to be one of the major factors for citrus decline in these states (Bhagabati *et al*, 1989; Chakroborty et al., 1993).

In the South and Central India, CTV is a chronic problem that occurs in mixed infections with huanglongbing (citrus greening) (Ahlawat, 1997). Recently, based on field survey, ELISA and PCR test, an overall CTV disease has been reported CTV in India; 26.3% in Central India (Maharastra), 47.1-56.0% in NE India (Assam, Meghalaya, Sikkim and Darjeeling hills), 36-50% in South India (Andhra Pradesh and Karnataka) and 16-60% in North-Northwest India (Uttarakhand, Delhi, Punjab, Rajasthan) (Biswas *et al*., 2014a).

3. TRANSMISSION AND DIAGNOSTICS OF CTV IN INDIA

Dispersal of CTV in new areas is taken place through virus infected bud woods or seedling plants and locally by insect brown citrus aphid (BrCA; *Toxoptera citricidus*).In India occurrence of BrCA is very common in most of the citrus growing areas in Northeast India (Ahlawat, 1997, Biswas, 2008). Diagnosis of CTV in field condition is difficult because, infected citrus trees do not necessarily produce diagnostic symptoms. Therefore, advanced biological and molecular diagnostic tools for detection of CTV have been developed and used.Electron microscopy, ELISA, PCR and sequencing are being used nowadays to accurately detect the virus time to time (Chakroborty *et al*., 1992; Ahlawat, 1997; Biswas, 2008; Kashyap *et al*., 2015) ELISA is a widely used method for rapid detection of CTV (Ahlawat *et al*., 1992; Biswas et al., 2008; Biswas et al., 2014b). The PCR using specific primers has been reported to be a sensitive, reliable and quick method for detection of CTV (Biswas, 2008, Biswas et al., 2012b). More than 30 pairs of PCR primers targeting different genomic region of CTV have been developed and used for detection of CTV (Biswas et al., 2012a).

4. SYMPTOMS, HOST RANGE, BIOLOGICAL INDEXING AND HOST RESISTANCE IN INDIA

First indication in occurrence of *Tristeza* or citrus decline disease in Indian subcontinent was given by Brown in 1920 in Peshawar (now in Pakistan) observing failure of Malta sweet orange on sour orange (*C. aurantium*) root stock. Citrus decline was reported in North India in during 1965 and in Bombay State in during 1968. It infects all the commercially grown citrus species, cultivars and hybrids of mandarin (*Citrus reticulata*), sweet orange (*C. sinensis*), Kagzilime/acid lime (*C. aurantifolia*), sweet lime/lemon (*C. limettoides / limon*) grown in India that has killed more than one million citrus trees (Ahlawat, 1997; Biswas, 2008). Mexican lime/Kagzi lime is commonly used as an indicator host and trifoliate orange is used to filter tristeza from mixed infection of other citrus viruses (Tanaka *et al*., 1971).

In India most citrus species and cultivars are susceptible to infection but some are tolerant and do not show obvious symptoms. Previously, Rough lemon (*C. jambhiri*) and trifoliate hybrid (Rangpur lime X *P. trifoliata*) were immune when tested in the Darjeeling hills (Biswas, 2008). However, Rough lemon and Rangpur lime were infected through inoculation by certain CTV isolates which show vein clearing and vein corking symptoms in the Darjeeling hills (Biswas et al., 2010). All the cultivated lime/lemons/Kagzilime, Assam lemon (*C. lemon*), Tahiti lime (*C. latifolia*) and Sweet lime) are highly susceptible to CTV (Biswas, 2012b). Earlier, it has been reported that mandarin is tolerant or resistant to CTV (Ahlawat, 1997). Howevers, except Kinnow mandarin, all the other cultivated mandarins (Darjeeling, Sikkim, Khasi, Nagpur, Mudkhed and Coorg mandarin) are infected by CTV in field and as well as in greenhouse graft transmission (Biswas, 2010, Biswas et al., 2012a, Singh et al., 2013; Sharma et al., 2011, Tarafdar et al., 2013). The Kinnow mandarin trees were free from CTV infection, whereas, Sweet orange trees orchard was highly susceptible to CTV (Sharma et al., 2011).

5. GENETIC DIVERSITY, DISTRIBUTION, INTRA-FARM DIVERSITY OF CTV IN INDIA

Genetic diversity and factors responsible for the origin of CTV variants in India have been examined by several Indian workers. Initially analyzing very few CTV isolates, three variants sharing 89-97% nt identity among them were reported from the Darjeeling hills, based on coat protein (CP) and

5′ORF1a (L-Pro domain) (Biswas, 2010). By analyzing several CTV isolates (114 CTV isolates; 16-19 of from central, 52-54 from Northeast, 17-18 from South and 14-15 North India (Table 1) it was observed that at least seven to nine CTV genotypes exist in citrus growing regions of India (Roy et al., 2005; Biswas, 2010; Sharma et al., 2011; Biswas et al., 2012a, Singh et al., 2013; Tarafdar et al., 2013; Vikas Chander et al., 2015).. Indian CTV isolates are extremely diverse sharing 80-99% nt identity among them for CP and 5′ORF1a. Several potential recombination events among Indian CTV isolates have been determined and recombination phenomena are responsible for evolution of divergent CTV isolates in India. Five CTV variants, VT (Kpg3), K5, T30, HA16-5, AG2 and ARI are existed Northeast citrus growing areas (Biswas et al., 2012b; Tarafdar et al., 2013). The variants AG2 and AR1 both are reported from Assam. Most of the Indian isolates are similar to the Israel severe CTV isolate VT genotype and this genotype is prevalent in all the citrus growing areas of India.

Individual farm in many citrus growing regions of India shows occurrence of divergent CTV isolates (Biswas, 2010; Biswas et al., 2012a; Sharma et al., 2011). A sweet orange farm at IARI, New Delhi and mandarin farm at IARI-Regional Station of the Darjeeling show three (VT, T36 and D13 types) and three CTV variants (VT, NZRB-G90 and B165), respectively.

Table 1: Sequence diversity and distribution of *Citrus tristeza virus* variants in India*

Geographical region	Based on 5'ORF1a(L-Pro domain)			Based on CP gene(ORF7)		
	No of isolate studied	% range of nt identity	CTV variant	No of isolate studied	% range of nt identity	CTV variant
Northeast India	54	83-98	Five (VT, K5, T30, HA16-5, AG2 & AR1)	52	88-99	Five (I, III, V, VI and VII))
North India	14	85-98	Three (VT, D13 and T36)	15	88-99	Three (I,V and VII)
Central India	20	91-99	Two (VT and T30)	16	92-99	Five (I, IV,V, VI and VII)
South India	17	86-99	Eight (AR1, VT, BAN 1, B165, HA16 -5, K5, D13 & T36)	18	88-99	Five (I, III, V,VII and VIII)
Overall India	105	78-99	Nine	101	86-99	Seven
Worldwide	-	77-99	Nine	-	86-99	Eight

*: Using the data from Biswas *et al*., 2012b; Tarafdar et al., 2013

6. COMPLETE CTV GENOME IN INDIA

Based on biological property and host range study, a CTV isolate Kpg3 of the mandarin growing areas of the Darjeeling hills was identified as a decline inducing strain (Biswas et al., 2012). The complete genome, 19253 nt in length, of isolate Kpg3 was sequenced, analyzed and submitted in NCBI database (Acc. No.HM 573451 (Biswas *et al*., 2012a). In phylogenetic relationship, the Kpg3 is closely related to Israel severe CTV isolate VT and it is a recombinant strain (Biswas *et al*., 2012a).

Further, 3' half of the genome (8398 nt) comprising ten genes (ORFs 2-11) of four other CTV isolates, B5 of Bangalore (HQ912023), D1 of Delhi (HQ912022), G28 of Assam (KJ914661) and Kat1 of Vidarbha (KJ914662) were sequenced and compared with other Asian and internationally recognized CTV genotypes. All the Asian isolates categorized into six genogroups, whereas the Indian isolates fell into four and other Asian isolates into three genogroups. Indian isolates B5, D1, Kat1 and Kpg3 grouped together (Kpg3Gr) along with Florida isolate T3. However, the isolate B5 was placed distantly from other members of Kpg3Gr. Thus isolate B5 might be a new isolate. The isolate G28 was found to be distinct lineage.

7. EFFORT FOR CROSS PROTECTION IN INDIA

Cross protection is the ability of mild strains or isolates to protect the severe or more virulent strains or isolates of the same virus. Cross protection has been successfully used in management of CTV in many countries like Brazil, Australia, Japan and South Africa (da Graça and van Vuuren, 2010; Roistacher et al., 2010). Cross protection of CTV was initiated in India during 1970 and some CTV strains were identified based on vector specificity (Capoor and Chakroborty, 1980). The mild cros protecting strain (MCPS) was identified and used to manage sever CTV in Tirupathi and Bangalore region (Balaraman and Ramakrishnan, 1977; 1980). Unfortunately, the experiment was failed probably due to appearance of severe strains those were mixed with the used mild strain.

Recently, effort was made to identify pure MCPS of CTV with the help of *in silico* molecular-based codon biasness analysis using CP gene of CTV isolates from Northeast India and their biological evaluation (Pal Choudhuri et al., 2016). Two Indian CTV isolates were identified as putative mild CTV type which are under evaluation challenging with severe strain (Pal Chaudhuri et al., 2016).

8. EFFORT FOR TRANSGENIC RESISTANCE TO THE VIRUS

Citrus transformation with various gene constructs including native and untranslatable version of coat protein and replicase genes has been successfully performed in many citrus species to confer resistance to CTV. The epicotyl of *in vitro* germinated seedling is used as the most responsive explant (Moore *et al.,* 1992). As p25 (CP gene) and p23 (ORF 1) act as suppressor of plant RNA silencing mechanism, for transformation of citrus, antisense and hairpin gene constructs were made targeting conserved sequence of p25 and p23 of CTV in pBin AR and pCAMBIA2301 binary vector (Tarafdar et al., 2009; Saha et al., 2014, Biswas unpublished). Transformation protocol of Kagzilime was developed using CTV constructs using epicotyl explant (0.75-1 cm) of *in vitro* grown 21-28 days old seedling (Tarafdar et al., 2009; Saha et al., 2014). However, Regeneration efficiency up to 1.38% was observed for cocultivated plant, whereas regeneration efficiency up to 84% was found control plant. Maximum number of five micro shoots/explant was regenerated at 2.0 mg/l BAP.

9. BUD WOOD AND SHOOT TIP GRAFTING FOR CTV FREE PLANTING MATERIALS IN INDIA

Virus diseases of citrus are managed by an integrated approach using virus free planting material, host resistance, sanitation, control of insect-vector, regulatory measures and some cultural practices. As citrus, is a vegetativlely propagated crop, use of virus-free planting material is essential. This can be achieved by careful indexing of budwood using sensitive diagnostic approaches. Citrus bud wood grafting and shoot tip grafting (STG) for production of disease free citrus planting materials are being practiced in many citrus growing areas in India (Vijaykumari et al., 2006; Biswas et al, 2009; Sanabam, et al., 2015). STG is more preferred to develop virus-free plantmaterial and it is an alternative to the use of nucellar seedling. Citrus plants produced through grafting did not show the long juvenile phase of plants compared to the plants obtained from nucellar seedling. The CTV-free mandarin (*Citrus reticulata* Blanco.) tree mother stocks was identified and grafted on Rangpur lime (*C. limonia*) and rough lemon (*C. jambhiri*) root stocks in the Darjeeling hills and disease free grafted mandarin plants were developed and supplied to the growers. Disease free Khasi mandarin plants were developed by STG on rough lemon root stock in Northeast India (Sanabam, et al., 2015).

10. CONCLUSION

The old citrus orchards, particularly in NE India, are severely infected by CTV. Therefore, sanitation and replantation of virus free citrus plants are prime factors for reduction of crop losses. The random distribution of CTV infected planting materials as a means of virus dissemination is common in India. Thus legislation or notification from the Government side is important to develop budwood certification programmes and supply disease-free seedlings to the growers. Efforts have been made to produce disease free planting materials through seed certification and shoot tip grafting and these are successfully practiced in new plantations in many citrus growing areas in India.

The technologies for detecting CTV in planting material by biological, serological and molecular methods have been perfected and are being utilized to detect virus at early stages to develop a long term disease management in India. Citrus orchards of many citrus growing areas of India, particularly in Northeast India, are being wiped out due decline or slow death caused by CTV. The genomics, complex population and geographical distribution of variants of CTV have clearly been understood in India that would help in understanding of disease epidemiology. Recombination between divergent CYTV isolates play major role for evolution of CTV variants. Molecular analysis of genomes of large number of CTV isolates of India determine occurrence of several CTV variants; some are distincts in India and new to the world. These studies would lead to formulate an improved diagnostics by development of specific primers targeting conserved sequence and further build up a molecular-based RNAi-mediated management strategy targeting conserved sequence for development of transgenic citrus plants. It is known that CTV complex represents a mixture of different virus strains, may be severe, mild or intermediate. Broad-spectrum mild strains are needed against multiple strains for practical applications of cross protection against CTV. The genes of interest have been cloned and transgene constructs were made for transformation of citrus to develop virus resistant transgenic plants.

REFERENCES

[1] **Ahlawat, Y.S.** 1997. Viruses, greening bacterium and viroids associated with citrus (Citrus species) decline in India. *Indian J. Agr. Sci.* **67**: 51-57.

[2] **Ahlawat, Y.S., Chakraborty, N.K., Varma, A.** and **Raychaudhari, S.P**. 1992. Diagnosis of diseases caused by viruses and fastidious prokaryotes in citrus in India. *Proc. Int. Soc. Citriculture* **2**: 749-751.

[3] **Anonymous**. (2012).Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses (Editors, Andrew M.Q. King, Michael J. Adams, Eric B. Carstens, and Elliot J. Lefkowitz), International Union of Microbiological Societies, Virology Division Amsterdam. 11 March **pp:** 871-879.

[4] **Balaraman, K.** and **Ramakrishan, K**. 1977. Studies on strains and strain interaction in *Citrus tristeza virus*. *Technical series bulletin University of Agricultural Sciences*, *Bangalore*. **pp** 62.

[5] **Balaraman, K.,** and **Ramakrishan, K.** 1980. Strain variation and cross protection in *Citrus tristeza virus* on acid lime. **In**: *Proc. 8th Conf. IOCV*. **Pp** 60-75.

[6] **Bar-Joseph M, Marcus R and Lee R F.** 1989. The continuous challenge of citrus tristeza virus control. *Annual Review of Phytopathology* **27:**291-316.

[7] **Bar-Joseph, M. and Dawson, W.O.** 2008. Citrus tristeza virus. Encyclopedia of Virology **pp.** 520-525.

[8] **Bhagabati, K.N., Ahlawat, Y.S., Chakroborty, N.K. and Borthakur, B.C.** 1989. Distribution of greening, tristeza amd mosaic disease of citrus in North eastern staes of India. *Indian Phytopath.* **42**: 552-555.

[9] **Biswas, K. K**., Tarafdar, A., Sharma, S. K. 2012b. Complete genome of mandarin decline *Citrus tristeza virus* of Northeastern Himalayan hill region of India: comparative analyses determine recombinant. *Arch Virol*, **157:** 579-583.

[10] **Biswas, K. K.** 2010. Molecular characterization of Citrus tristeza virus isolates from the Northeastern Himalayan region of India. *Arch Virol,* **155:** 959-963

[11] **Biswas, K. K**., Tarafdar, A., Diwedi, S. and Lee, R.F. 2012a. Distribution, genetic diversity and recombination analysis of Citrus tristeza virus of India. *Virus Genes*, **45**: 139-148.

[12] **Biswas. K. K**. 2008. Molecular diagnosis of *Citrus tristeza virus* in mandarin (*C. reticulata*) orchards of Darjeeling hills of West Bengal. *Indian J. Virol.* **19:** 26-31

[13] **Biswas**, K. K., Tarafdar, A., Jayakumar, B. K. and Pun. K. B. 2009. Strategy for the production of *Citrus tristeza virus*-free mandarin (*Citrus reticulata*) planting materials in the Darjeeling hills of India. *Indian Phytopathology* **62:** 376-80.

[14] **Biswas, K. K.,** Tarafdar, A., Sharma, S. K., Singh, J.K., Dwivedi, S., Biswas, K. and Jayakumar, B.K. 2014a. Current status of *Citrus tristeza virus* incidence and its spatial distribution in citrus growing geographical zones of India. *Indian Journal of Agricultural Sciences* **84:** 8-13.

[15] **Biswas**, K. K., Godara, S. and and Nayak, D. 2014b. Distribution of *Citrus tristeza virus* in the Darjeeling hills and their biological symptoms in mandarin orchards. *Indan J. Hort.* **71:** 408-411

[16] **Capoor, S.P.** and **Chakraborty N.K.** 1980. Further studies of citrus seedling yellow yellows in Deccan trap country. *Indian J. Hort*. **37**: 97-100.

[17] **Chakraborty, N.K., Ahlawat, Y.S., Varma, A., Chandra, K.J., Ramapandu, S.** and **Kapur, S.P.** 1992. Serological reactivity in *Citrus tristeza virus* strains in India. *In: Proc. 12th Conf. IOCV*. **pp:** 108-112.

[18] **Chandra, Vikash., Godara, S., Kumar, A., Saha, B. and Biswas**, **K. K.** 2015. Nucleotide sequence of ORF 11-p23 suppressor gene of *Citrus tristeza virus* isolate of different citrus growing states of India and determination of extensive genetic diversity. *J. Mycopathol Res.* **53 (1):** 25-29.

[19] **da Graça,J.V.,andvanVuuren,S.** P. 2010."Managing *Citrus tristeza virus* losses using cross protection," in *Citrus Tristeza Virus Complex and Tristeza Diseases*, A.V.Karasev, and M.E.Hilf (Eagan: APSPress), 247–260.

[20] **Ghosh, S.P.** (2007). Citrus fruits. ICAR, New Delhi.

[21] **Karasev, AV, Boyko, VP, Gowda S, Nikolaeva O V., Hilf, M E, Koonin, EV, Niblet CL, Cline, K, Gumpf, D J and Lee, R F.** 1995. Complete sequence of the citrus tristeza virus RNA genome. *Virology* 208-511-520.

[22] **Kashyap, A., Nath, P.D., Acharjee, S. and Biswas.K.K.** 2015 .Prevalence of *Citrus tristeza virus* in North Eastern region of India and molecular characterization of its isolates *Indian J. Hort.* **72(2):** 206-211

[23] **Melzer, M.J., Borth, W.B, Sether, D.M., Ferreira, S., Gonsalves, D. and Hu, J.S.** 2010. Genetic diversity and evidence for recent modular recombination in Hawaiian Citrus tristeza virus. *Virus Genes* **40**: 111-118.

[24] **Moore, G.A., Jacono, C.C., Neidigh, J.L., Lawrence, S.D.** and **Cline, K.** 1992..*Agrobacterium*-mediated transformation of Citrus stem explants and regeneration of transgenic plants. *Plant Cell Reports* **11:** 238-242.

[25] **Moreno, P., Ambros, S., Albiach-Marti, M.R., Guerri, J. and Pena, L. 2008.** Citrus tristeza virus: a pathogen that changed the course of the citrus industry. *Mol Plant Pathol* **9**:251-268.

[26] **Palchoudhury, S., Biswas, M. K.** and **Biswas, K. K.** 2016. Codon biasness: a fingerprint of *Citrus tristeza virus* in detecting mild cross protecting strain for disease management**. In**: ISMPP- 37th Ann conf and Nat Symposia on "Food security through Plant Health Protection", RAU, Pusa Samastipur, April 4-6, 2016, **pp**50.

[27] **Roistacher,C.N., daGraça,J.V.,and Müller,G.W. 2010.** Crossprotection against *Citrustristezavirus*-a review. **In:** *roceeding of Conferenceof the International OrganizationofCitrusVirology,17th.* eds M.E.Hilf,L. W.Timmer,R.G.Milne,andJ.V.da Graça(Riverside,CA:IOCV),1–27.

[28] **Roy, A., Manjunath, K.L. and Brlansky, R.H.** 2005. Assessment of sequence diversity in the 5-terminal region of Citrus tristeza virus from India. *Virus Res*. **113**: 132-142.

[29] **Saha, Bikram., Palchoudhury, S., Godara, S. and Biswas, K.K.** 2014. Development of gene construct targeting coat protein and supresor gene of *Citrus tristeza virus* gene and Transformation of citrus plant. In: National seminar on *"Advances In Plant And Microbial Research"* December, 12-13, 2014, Organized by Dept of Botany, University Of North Bengal, Siliguri-734 013, West Bengal **pp**38

[30] **Sanabama, R., Singha, N. S., Handiqueb, P.J. and Devia, H.S..** 2015. Disease-free khasi mandarin (Citrus reticulata Blanco) productionusing in vitro microshoot tip grafting and its assessment usingDAS-ELISA and RT-PCR. *Scientia Horticulturae*, **189**: 208-213.

[31] **Satyanayanana, T., Gowda, S., Mawassi, M., Albiach-Marti, M.R., Ayllon, M.A., Robertson, C., Garnsey, S.M and Dawson, W.O.** 2000 Closterovirus encoded HSP70 homolog and p61 in addition to both coat proteins function in efficient virion assembly. *Virology* **278**:253–265.

[32] **Sharma, S. K., Tarafdar, A., Khatun, D., Kumari Sumita and Biswas K. K..** 2011. Intra-farm diversity and evidence of genetic recombination of *Citrus tristeza virus* isolates in Delhi region of India. *Journal of Plant Biochem and Biotechnogy,* **21:** 38–43.

[33] **Singh, J. K., Tarafdar, A., Sharma, S.K., Biswas, K. K.** 2013. Evidence of recombinant *Citrus tristeza virus* Isolate Occurring in Acid Lime cv. Pant Lemon Orchard in Uttarakhand Terai

Region of Northern Himalaya in India. *Indian J. Virol*. **24**: 35-41.

[34] **Tanaka, H., Yamada, S. and Nakanishi.** 1971. Approach to eliminating tristeza virus from citrus trees by using trifoliate orange seedlings. *Bull. Hort. Res. Sta. Japan Sec.* **11**: 157-163.

[35] **Tarafdar, A., Shruti Godara, Sumita Dwivedi and Biswas, K. K.**. 2013. Characterization of *Citrus tristeza virus* and determination of genetic variability in North-east and South India *Indian Phytopath*. **66**: 302-307.

[36] **Tarafdar,A., Sumita Kumari and K. K. Biswas.** 2009. Agrobacterium mediated transformation of *Citrus aurantifolia* Kagzi lime using coat protein gene of *Citrus tristeza virus* **In:** *Proceeding of 5th International Symposium on Plant Pathology in the globalized era Indian Phytopathological society,* Indian Agricultural Research Institute, New Delhi, **pp** 390.

[37] **Vijaykumari, N., Ghosh, D. K., Das, A.K., Singh, A. and Singh, S.** 2006. Elimination of citrus tristeza virus and greening pathogens from exotic germplasm through *in vitro*shoot tip grafting in citrus. *Ind J. Agric Sci,* **76**: 209-10.