

Present status of *Citrus tristeza virus* infecting *Citrus* spp. in Darjeeling hills and its detection in different plant parts

Amalendu Ghosh · Amrita Das · K. B. Pun ·
R. Kumar · Ramu Meena · V. K. Baranwal

Received: 3 September 2013 / Accepted: 2 December 2013
© Springer Science+Business Media Dordrecht 2014

Abstract Darjeeling hills of West Bengal (India) are well known for the production of mandarin orange (*Citrus reticulata*). The spread of *Citrus tristeza virus* (CTV) in this area has threatened the cultivation of citrus. Therefore, a study of the incidence and distribution of CTV in Darjeeling hills was undertaken and a geographical map was prepared. In DAS-ELISA, a high titre of CTV was recorded in the tender shoot bark, leaf petiole and mid-rib of seven *Citrus* spp. Three citrus species, viz., pomelo, trifoliate and kumquat, were found to be free of CTV infection when tested in double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) and reverse transcription polymerase chain reaction (RT-PCR).

Keywords *Citrus reticulata* · Distribution · India · Mandarin orange · *Tristeza* · CTV · Darjeeling

A. Ghosh · A. Das (✉) · K. B. Pun · R. Kumar · R. Meena
Indian Agricultural Research Institute, Regional Station,
Kalimpong 734 301 West Bengal, India
e-mail: amritapatho@gmail.com

V. K. Baranwal
Advanced Centre for Plant Virology, Indian Agricultural
Research Institute,
New Delhi 110 012, India

Present Address:
K. B. Pun
Regional Rainfed Lowland Rice Research Station, Central
Rice Research Institute,
Gerua 781 102 Assam, India

Present Address:
R. Kumar
Indian Agricultural Research Institute, Regional Station,
Karnal 132 001 Haryana, India

Introduction

Citrus tristeza virus (CTV) is an economically important virus in all citrus-growing areas throughout the world, destroying millions of citrus trees over the last 70 years (Bar-Joseph & Dawson, 2008). CTV belongs to the *Closterovirus* genus of family *Closteroviridae*. It is a phloem-limited virus. The genome consists of a positive sense single strand RNA comprising 19,296 nucleotides and organized into 12 open-reading frames (Bar-Joseph *et al.* 1989; Karasev *et al.* 1995; Ruiz-Ruiz *et al.* 2007). Darjeeling hills of West Bengal are well known for the production of mandarin orange (*Citrus reticulata* Blanco), which fetches high export earnings to citrus growers. However, CTV causes severe loss in mandarin production in Darjeeling hills (Biswas *et al.* 2004; Mukhopadhyay *et al.* 1986). Earlier molecular diagnosis of CTV and sequencing of the complete genome of the Kpg3 isolate were reported from this region (Biswas 2008; Biswas *et al.* 2012), but no definite information on incidence and distribution of CTV in this area was available. In this work, we studied the distribution of CTV in mandarin orchards of Darjeeling hills. Effort was also expended to determine the best tissue type for detection of CTV through double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) and CTV-free *Citrus* spp.

Materials and methods

Citrus-producing areas of Darjeeling hills were surveyed during the winter season of 2011–2013. Mandarin plants 12–15 years old in different orchards were selected randomly for collection of leaf and bark samples. A total of