Indian J. Virol. (July–September 2013) 24(2):227–234
DOI 10.1007/s13337-013-0159-7

ORIGINAL ARTICLE

Highly efficient immunodiagnosis of Large cardamom chirke virus using the polyclonal antiserum against *Escherichia coli* expressed recombinant coat protein

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Received: 14 June 2013 / Accepted: 17 August 2013 / Published online: 1 September 2013

Abstract Large cardamom chirke virus (LCCV), genus *Macluravirus*, family *Potyviridae* is an important constrain in large cardamom production in India. Purification of LCCV from large cardamom tissues is difficult and therefore immunodiagnostic reagents are not available. In the present study, we have successfully expressed coat protein (CP) gene of LCCV in *Escherichia coli.* The purification of expressed protein by Ni-NTA affinity chromatography was inefficient due to precipitation of protein during renaturation. We have optimized a simple, inexpensive and efficient method for purification of the expressed CP through gel extraction with 5 % SDS followed by renaturation in Milli-Q water, which resulted in high yield (4.7 mg/ml) and good quality of the protein. A higher titer (1:256,000) polyclonal antibody (PAb) to the recombinant CP was produced, which strongly recognized LCCV in crude leaf extract and showed minimal background reaction with the healthy leaf extract in enzyme linked immunosorbent assay (ELISA) and dot immunobinding assay (DIBA). The sensitivities of the ELISA and DIBA were 5 and 0.1 ng of expressed protein, respectively. Both the ELISA and DIBA were validated with 100 % accuracy in detecting LCCV in field samples. The PAb differentiated *Cardamom mosaic virus*, another close relative of LCCV. Our study is first to report highly efficient immunodiagnosis with PAb to *E. coli* expressed recombinant CP of a virus under the genus *Macluravirus.* The antigen expression construct and PAb developed in the present study will be useful in production of virus free planting materials of large cardamom.

Keywords Large cardamom chirke virus · LCCV · Coat protein gene expression · ELISA · DIBA

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Introduction

Large cardamom (*Ammomum subulatum*) is cultivated as a cash crop under the shady and moist environment of eastern Sub-Himalayan Mountains. Sikkim state and Darjeeling district of West Bengal are the major large cardamom producing areas in India, where the crop is vital to the rural livelihood security. Large cardamom production is affected by two chronic viral diseases known as chirke and foorkey [14, 17]. Chirke disease characterized by streak mosaic symptoms on leaves was known to occur since 1958 [14]. Chirke disease causes gradual decline in plant growth and results in significant yield loss in the third year of infection [14–16]. Large cardamom chirke virus (LCCV), a new virus species under the genus *Macluravirus,* family *Potyviridae* was identified as the virus associated with the chirke disease [10]. LCCV, a (+)ssRNA virus is vectored by aphids, *Rhopalosiphum maidis, Brachycaudus helichrysi* and *Myzus persicae* in a non-persistent manner [10, 15].

Large cardamom is predominantly grown through vegetative propagation of suckers. Although, several aphid vectors are known, LCCV primarily spreads through planting material. For propagation of virus free planting material of large cardamom, sensitive diagnostic reagents and methodology are necessary. Purification of LCCV