Research Note :

Development of gene construct of conserved *rep* gene from tomato leaf curl virus from natural host plant for resistance against tomato leaf curl virus

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The tomato is grown worldwide for its edible fruits, with thousands of cultivars having been selected with varying fruit types. India ranks 4th in tomato production in the world. It produces about 10.2 million tones tomato annually from about half a million hectares (FAOSTAT, Crop statistics, 2008). Tomatoes are now eaten freely throughout the world, and their consumption is believed to benefit the heart among other things. They contain lycopene, one of the most powerful natural antioxidants. Tomato consumption has been associated with decreased risk of breast cancer, head and neck cancers and might be strongly protective against neurodegenerative diseases. (Zhang *et al*., 2009). There are over 10 important viruses which naturally infect tomato and some of these viruses have a large number of distinct strains. The most destructive viruses affecting tomato are Tomato leaf curl virus (ToLCV) causing tomato leaf curl disease (ToLCD), whose occurrence in India has been known since 1948 (Varma and Malathi, 2003). Efforts to develop tomato varieties resistant to ToLCV by traditional breeding have not been successful, as natural sources of resistance are not available. The virus is transmitted by whiteflies (*Bemisia tabaci)* that are attracted to young leaves and growing tips. The virus is not transmitted mechanically nor via seed. The concept of pathogen-derived resistance was introduced in plant virology based on the report that transgenic tobacco expressing tobacco mosaic virus (TMV) coat protein showed a resistance to TMV infection. This type of resistance generally referred to as coat protein-mediated resistance, has been described for many virus/host systems, but is restricted to the virus closely related to the expression of functional or altered introduced viral replicase gene (Powell-Abel *et al*., 1986). Viral gene suppression involves a coordinated series of sub cellular events that ultimately lead to the post-transcriptional silencing of gene expression. This phenomenon has been termed as co-suppression or post-transcriptional gene silencing, which operates through homology-dependent gene silencing. Various models have been proposed to demonstrate the mechanism of virus resistance and PTGS of endogenous gene in transgenic plants, containing sense or antisense transgenes. The Cauliflower Mosaic Virus (CaMV) 35S Promoter is the most well-known and widely used promoters in genetics research. One of the reasons is that it was one of the earliest promoters discovered, and it is also a *constitutive* promoter, meaning that it is always "on" - it makes the gene it is linked up to express constantly (Baulcombe 1996).

Five leaf curl infected samples of tomato plants were collected from Indian Institute of Vegetable Research Varanasi, U.P. Out of those five samples two were found positive through electron microscopy. The DNA was isolated from one positive plant's leaf sample by DNeasy Plant Maxi Kit (QIAGEN Ltd.). Three sets of PCR primers were tried to get the sequence from the *rep* gene out of which primer, Forward Primer 'catcaagatctgt-ggagagagc' Reverse Primer 'tagacgagacc-caatcgacg' are able to amplify *rep* gene. and sequence confirmed by sequencing. The condition was set for PCR as initial denuration 94°C for 5 min, 94°C for 30 sec, 60°C for 40