

# Functional analysis of $\beta$ C1 protein encoded by *Tomato leaf curl Java virus*, a monopartite begomovirus

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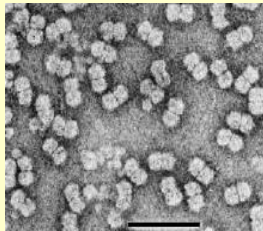
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We have isolated and cloned tomato leaf curl Java virus (ToLCJAV, 2754nt) and satellite DNA molecule (DNA $\beta$  1354nt)<sup>1</sup> from tomato plants exhibiting leaf curl symptom from Java, Indonesia. We found that ToLCJAV alone could infect plants systemically when co-inoculated with satellite DNA induce very severe leaf curling. when  $\beta$ C1 gene was mutated (frame shift) & co-inoculated with cognate DNA, leads to decrease symptoms drastically. We found that  $\beta$ C1 gene functions as pathogenicity determinant, suppressor of PTGS & localized towards cell periphery.

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ToLCJAV symptoms in tomato plant.



Particles of Gemini virus as shown in E.M (Bar=100nm)

Tomato leaf curl disease is among the most important limiting factors that affect tomato production in world wide including south East Asia. Recently, we have isolated tomato leaf curl Java virus shown to be associated with a novel signal stranded DNA satellite. Post transcriptional gene silencing (PTGS) in plants is a natural defense response against virus infections, and as a counter defense strategy, viruses have evolved to encode silencing suppressor proteins. For systemic infection, the virus moves cell-to-cell from the site of inoculation to vascular tissue and via phloem to other plant tissues. To move, viral DNA has to shuttle in and out of the nucleus and through plasmodesmata. In the present study, we have identified functions encoded by  $\beta$ C1 proteins encoded by ToLCJAV, which poses a serious threat to agricultural production in Indonesia.

## METHODOLOGY

We constructed infectious clones of ToLCJAV and DNA $\beta$ mC1 in binary vector pBI121 & agro-inoculated to *N. benthamiana*. The  $\beta$ C1 gene and were expressed from the *Potato virus X* (PVX)<sup>2</sup> vector under the control of the 35S promoter, inoculated to *N. benthamiana* 16C plants for their capacity to produce PTGS. For subcellular localization, ORFs were cloned at 5' end of pGFP(S65T)3 & bombarded onto epidermal cells of *N. benthamiana* & Onion cells, observed by using confocal microscopy

## RESULTS

*N. benthamiana* plants agro-inoculated with ToLCJAV & satellite DNA $\beta$  developed severe downward leaf curling & stunting. By contrast, when co-inoculated with mutated  $\beta$ C1 gene drastically reduced symptoms (Fig1). RNA silencing is a major means of defense against viruses. For PTGS assay, when PVX $\beta$ C1/PVXGFP transgene were co expressed in 16C leaves showed downward curling and maintained the green fluorescence under UV light while PVXm $\beta$ C1/PVXGFP transgene showed systemic silencing spread of silencing (Fig2). Furthermore, the levels of GFP mRNA and siRNA of infected plants were detected and analyzed by northern blotting (Fig2). The results of subcellular localization showed that  $\beta$ C1 is localized in the cell periphery (Fig3)

Expression of the  $\beta$ C1 gene in transgenic plants also induces developmental abnormalities (unpublished work). It is likely that the  $\beta$ C1 protein may be involved in the regulation of miRNA involved in plant development. The  $\beta$ C1 and AC4/C4 proteins share characteristics of pathogenicity and silencing suppression. The precise mechanism of action of the  $\beta$ C1 protein will be the focus of our future research activities

## Conclusion

1)  $\beta$ C1 gene function as pathogenicity determinant, strong suppressor of PTGS & localized towards cell periphery .

## Acknowledgement

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## References

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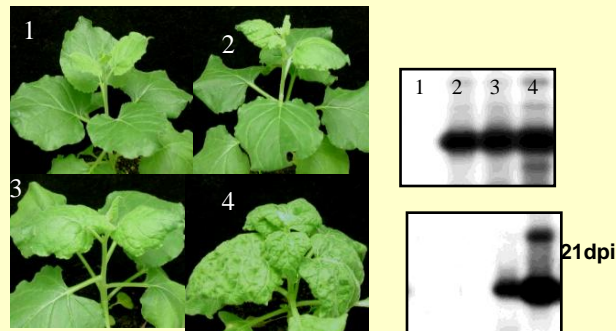


Fig 1. Agro-inoculation (1)Mock, (2) ToLCJAV, (3) ToLCJAV + m $\beta$ 02C1, (4) ToLCJAV + DNA $\beta$ 02. Right side southern blot PVX-GFP/m $\beta$ 02C1

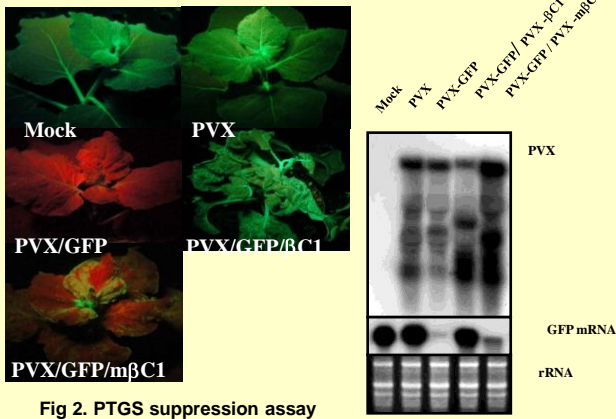


Fig 2. PTGS suppression assay

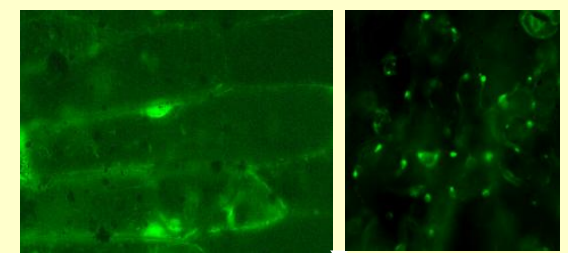


Fig3 Subcellular localization of  $\beta$ C1 protein encoded by DNA betasatellite