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Virus-induced gene complementation in tomato

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Virus-induced gene complementation (VIGC), a plant virus technology based on *Potato virus X* for transient overexpression of endogenous genes complemented tomato mutants, resulting in non-ripening fruits to ripen. This efficient "gain-of-function" approach involves no stable transformation, and reveals a fruit-specific transcriptional network that may exist among key transcription factors in modulating tomato ripening. Thus, VIGC represents a novel and feasible strategy for gene functional analysis in plants.

Keywords: plant virus technology, functional complementation, VIGC, ripening inhibitor, colourless non-ripening, *Potato virus X*

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Plant virus technology including small interfering RNA-mediated virus induced transcriptional and posttranscriptional gene silencing (Sir VIGS), microRNA-mediated VIGS (Mr VIGS), virus-based RNA mobility assays, and virus-based ectopic gene expression, is an important forward and reverse genetic approach to delineate gene function in plants. In particular, Sir VIGS has been widely used in many plant species, owing to its capability of effective and rapid gene "knock-down."[1,2] A drawback for Sir VIGS is the off-target silencing, and it can cause nonspecific mRNA degradation or translation repression.[3-5] This disadvantage can be overcome to a certain degree by Mr VIGS in which only one known mature microRNA can be expressed from a specifically designed microRNA precursor by a virus vector, leading to highly specific silencing of target genes.[6] On the other hand, solid evidence for illuminating biological significance of a given gene often involves "gain-of-function" analysis, especially phenotypic complementation in mutant background. This is usually performed through labor-intensive and time-consuming stable plant transformation.

However, considerable progress has been made to test the feasibility of plant virus technology as a gene overexpression system. For instance, expression of heterologous genes from recombinant viral vectors results in a high level of foreign proteins in plants.[7-9] This implies that a viral transient expression system could be applied to express endogenous genes for rapid induction of specific phenotypes and phenotypic complementation, thus as a "gain of function" tool to define plant gene functions and bridge knowledge gaps between genotypes and phenotypes.

To exploit whether virus technology could be indeed used in a "gain-of-function" assay, we have developed a virus-induced gene complementation (VIGC) system from a modified *Potato virus X* (PVX) vector. Viral ectopic overexpression of the MADS-box transcription factor (TF) gene *SlMADS-RIN*, involving no stable tomato transformation, was able to complement non-ripening phenotype in tomato *Ripening inhibitor* (*rin*) mutant.[10] To further test the usefulness of the VIGC technique, we expressed the SBP-box SlSPL-CNR TF in the tomato *Colorless non-ripening* (*Cnr*) mutant fruit, and found that viral delivery of a functional *SlSPL-CNR* gene caused complementation

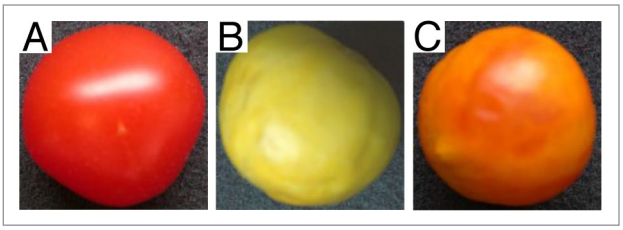

Figure 1. VIGC-mediated complementation of ripening in *Cnr* mutant fruit. (**A**) Wild-type tomato (*Solanum lycopersicum* Ailsa Craig) ripened normally. (**B**) *Cnr* mutant fruit in Ailsa Craig background remained colorless non-ripening. (**C**) Complementation of ripening occurred in *Cnr* mutant fruit treated with a VIGC vector PVX carrying a functional *SlSPL-CNR* gene.

of the *Cnr* mutant (**Fig. 1**). Moreover, comparative gene expression analysis of ripening and non-ripening tissues at the precisely equivalent growing stage collected from same individual fruits, revealed that SlMADS-RIN is a master TF in a transcriptional network in modulating tomato fruit ripening.[10] This model is supported by the fact that SlMADS-RIN directly interacts with promoters of several key ripening-associated TFs.[11,12]

Taken together, our results indicate that the efficient VIGC technology represents a novel strategy to reveal gene functions in tomato. Considering the availability of a wild range of plant virus-based gene expression vectors, VIGC technique based on different plant viruses can be developed for functional genomics in diverse plant species including many economically important crops.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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