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News and Views

ViPariNama: combining CRISPR and systemic virus-based vectors for rapid phenotyping of complex plant traits

Bernarda Calla [1] **and Javier Edgardo Moreno** [2]

1 Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA
2 Instituto de Agrobiotecnología del Litoral (UNL-Conicet), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe 3000, Argentina

A breeder's panacea would be to manipulate plant traits to their "gusto e piacere" (taste and pleasure). Tolerance to drought and salinity, resistance to pests and pathogens, selective resistance to herbicides, increased yield through timed flowering, and better nutritional or productive traits are some of the attributes crop breeders continuously aim to improve. The development of recent genome editing tools, most notable CRISPR/Cas9, promises faster and less expensive crop breeding. Many traits, including plant height and flowering time, are controlled by complex, multiloci genetic programs with both activation and repression mechanisms to tune plant development (Eshed and Lippman, 2019). Thus, there is a need for tools that allow rapid evaluation of spatial and temporal phenotypic outcomes resulting from gene expression level reprogramming.

In this issue of *Plant Physiology*, Khakhar *et al.* (2021) report a system called ViPariNama (ViN) that accelerates phenotypic assessment of genetic changes by minimizing the use of stable transgenic plants. The ViN system exploits the customizable DNA recognition, cutting, and scaffolding properties of Cas9 and single-guide RNAs (sgRNAs), together with the ability of RNA viral vectors, to quickly spread in the plant and persist for extended periods of time. ViN seeks to alter plant attributes by subtly manipulating gene expression levels of transcription factors. As a proof of concept, Khakhar and colleagues demonstrated altered growth and leaf-pigmentation effects in *Nicotiana benthamiana*, Arabidopsis (*Arabidopsis thaliana*), and tomato (*Solanum lycopersicum*) plants.

The authors carried out a series of experiments to demonstrate the capabilities of the ViN 1.0 system. They used transgenic *N. benthamiana* plants constitutively expressing a gibberellin biosensor consisting of inactive Cas9 (dCas9) fused to a gibberellin (GA) degron (a targeting sequence for degradation in the presence of GA) and to a repressor domain (Khakhar et al., 2018). ViN vectors carrying sgRNAs designed to bind to the upstream regulatory region of five putative GA 20-oxidase (*GA20ox*) gibberellin biosynthesis genes (Spielmeyer et al., 2002) were delivered via Agrobacterium infiltration of young leaves of the transgenic *N. benthamiana*. The delivery of the customized ViN vectors into the plant was sufficient to trigger the recruitment of the biosensor to the upstream regulatory region of at least two *GA20ox* genes and repress their transcription, lowering GA levels and resulting in smaller leaves. ViN 1.0 was further tested using Arabidopsis plants with constitutive expression of an active Cas9 fused to an RNA-binding protein and a regulatory domain. These plants were infiltrated with a sgRNA to direct the regulatory activity of Cas9 to repress the expression of the three *GIBBERELLIN-INSENSITIVE DWARF 1* genes (*GID1a*, *GID1b*, and *GID1c*) that encode GA receptors while activating the expression of PAP1, a positive regulator of anthocyanin accumulation in the plant (Griffiths et al., 2006). The transgenic plants were consequently smaller and contained more anthocyanin in their leaf tissues.

With the idea of increasing the flexibility of the ViN system, the authors designed ViN 2.0. In this second system, only Cas9 protein (either active or inactive) needs to be stably transformed in the plant with all other components delivered by ViN vectors; at least one of these ViN vectors must contain the sgRNA and another must contain an RNA-binding protein fused to an activator or repressor regulatory domain. This strategy allows phenotypes to be easily tuned with a mix and match of protein effectors and

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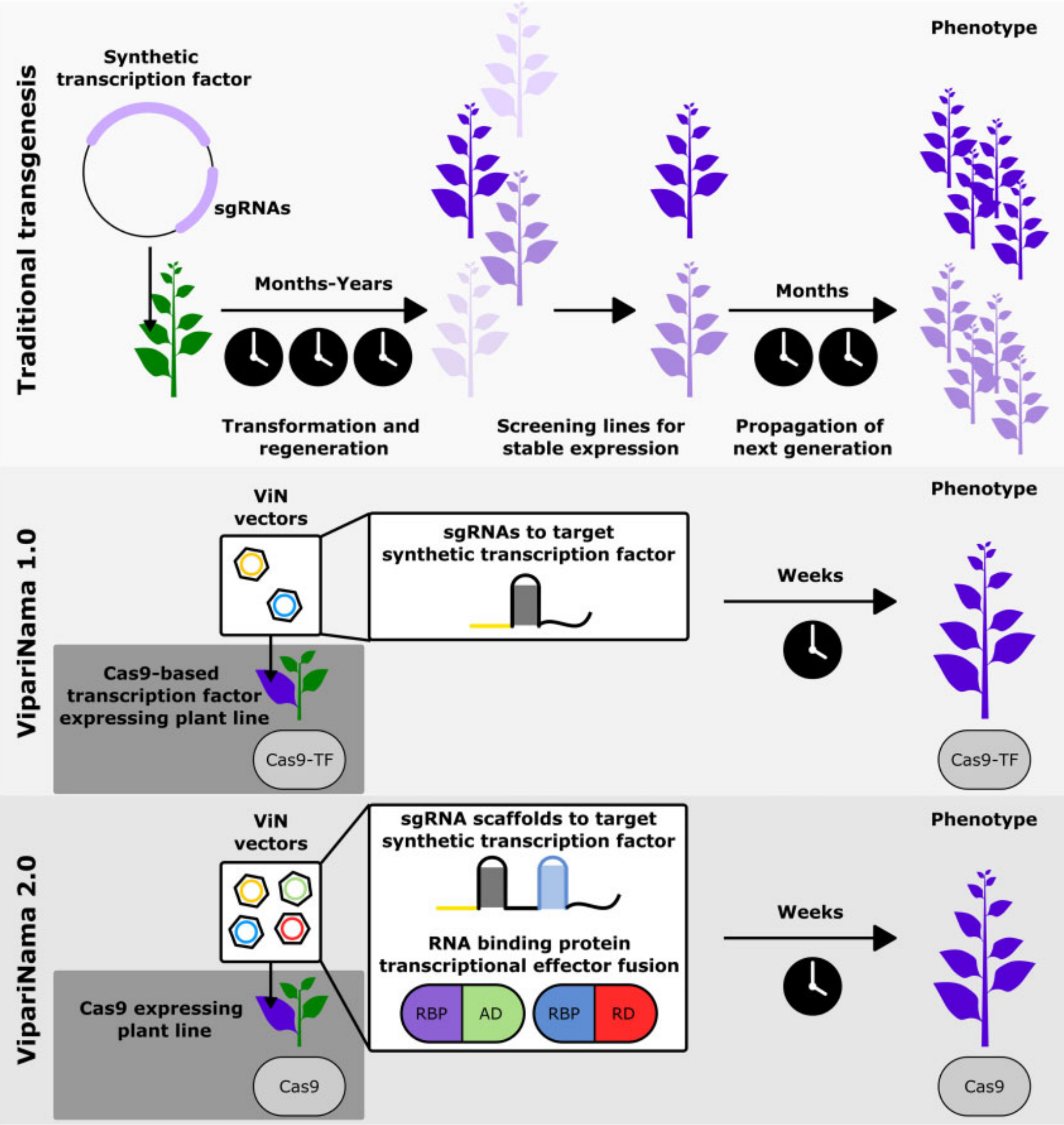

Figure 1 ViN offers a tool to accelerate plant phenotyping. The ViN vector system was tested to induce rapid effects on plant growth and metabolism. This graph compares the pipeline of traditional transgenesis (top), ViN 1.0 (middle), where ViN vectors deliver sgRNAs to a transgenic plant expressing Cas9-based transcription factors, and ViN 2.0 (bottom), using multiple ViN vectors to deliver sgRNA scaffolds and an RNA binding effector to a Cas9-expressing plant. RBP, RNA-binding protein; AD, activating domain; RD, repressing domain; TRV, Tobacco Rattle Virus (Figure from Khakhar et al., 2021).

sgRNAs. For these synthetic transcription factors to be active, the vectors containing the sgRNAs and the effector must colocalize within the same cell. To ensure colocalization, Khakhar and collaborators also introduced a short tRNA-like motif derived from the Arabidopsis *FLOWERING LOCUST T* known to improve RNA systemic mobility in the plant (Li et al., 2009). ViN 2.0 was tested in several settings demonstrating its ability to allow easy swapping of effectors and targets and to regulate multiple genes. In addition, the system was tested for its systemic mobility by measuring temporal and spatial gene expression levels and vector abundance.

As a final proof of concept, and to move beyond model plants into crops, the ViN 2.0 system was tested in tomato. Tomato plants with Cas9 integrated in the genome were Agro-infiltrated with ViN vectors carrying a functional N-terminal fragment of the TOPLESS transcriptional repressor from Arabidopsis and sgRNAs targeting the promoter region of the gene coding for PROCERA, the single DELLA protein in tomato. The delivery of the ViN vectors resulted in a reduction of the DELLA protein in the plant and concomitant increase in internode length.

Generating improved crop cultivars can take several years. Modern technologies derived from next-generation sequencing, accelerated breeding strategies (Watson et al., 2018), and precise genome engineering, such as CRISPR/Cas9, have the capability of accelerating breeding programs. However, phenotyping, a critical step in crop improvement, is still

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slow since it is affected by both genotype and environmental variables. The viral-vector-based systems developed by Khakhar et al. demonstrate possibilities to complement others derived from the synthetic CRISPR-based transcriptional regulators toolbox (Zalatan et al., 2015). The effectiveness of infiltrating viral-derived vectors comes from their ease of introduction (by leaf infiltration) combined with their capacity of systemic spread in the plant (Figure 1). Although the ViN systems still depend on a transgenic plant expressing different versions of Cas9, and/or assemblies of effectors and repressors, they represent a step towards the availability of a fully customizable tool for fast phenotypic evaluation. Strategies that do not depend on integration of Cas9 into the plant genome would introduce possibilities for a new generation of tools to study and engineer crops.

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