pLX vectors and JoinTRV simplify plant virus engineering and heritable CRISPR/Cas9 editing of plant genomes

Fabio Pasin[1,2,†,*], Verónica Aragonés[1], Flavio Aliaga[1,3], José-Antonio Darós[1,*]

[1] Institute of Molecular and Cellular Biology of Plants (CSIC - UPV), Valencia, Spain

[2] School of Science, University of Padova, Padova, Italy

[3] Dirección de Desarrollo Tecnológico Agrario, Instituto Nacional de Innovación Agraria (INIA), Lima, Peru

[†] Former address: Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan

* Correspondence: F.P. <f.pasin@csic.es>, J.-A.D. <jadaros@ibmcp.upv.es>

ABSTRACT

Plant viruses have been exploited in multiple biotechnology and synthetic biology applications as varied as biopharmaceutical production, flowering induction and accelerated plant breeding, transient crop reprogramming, or heritable mutagenesis of crop genomes.[1–4]

Viral vectors allow delivery of CRISPR/Cas components into human and plant cells, and to recover cells with mutations at the desired genomic loci. Recent advances for crop trait engineering include the use of viral vectors for multiplexed single-guide RNA (sgRNA) delivery into Cas-expressing transgenic hosts, for delivery of Cas nucleases and sgRNAs, and for delivery of cell-to-cell mobile sgRNAs.[5–11] Generation of virus infectious clones and viral vectors is nonetheless a major bottleneck that can limit applications of virus-derived tools for plant cell reprogramming and crop trait engineering.

To streamline assembly of plant virus construct and viral vector engineering, we developed pLX, a set of mini binary T-DNA vectors (~3 kb) suitable for advanced cloning methods.[12] They have allowed one-step assembly of infectious clones of RNA and DNA viruses which were successfully delivered into plants by *Agrobacterium*-mediated inoculation.[13] Synthetic genomics approaches facilitate flexible hypothesis testing and rapid product development. To assist their adoption by plant virologists and biotechnologists we conceived SynViP, a rationally designed synthetic genomics framework with plant virome capacity. The framework can be used with linear and circular DNA molecules, does not require subcloning steps, and is insensitive to fragment terminal sequences. SynViP was successfully used to rescue a genuine plant virus based on a digital template.[14]

We further developed JoinTRV, a novel plant expression system based on pLX and tobacco rattle virus. JoinTRV consists of a single *Agrobacterium* clone co-transformed with two compatible mini binary vectors from which the TRV RNA1 and an engineered version of TRV RNA2 are expressed. Our system allowed recombinant protein production in plants, and robust virus-induced gene silencing (VIGS) of endogenous transcripts using bacterial suspensions at very low optical densities. JoinTRV-mediated delivery of sgRNAs in Cas9 transgenic hosts allowed somatic cell editing efficiencies of ~90%, as well as the visual detection of somatic cells with functional loss of the targeted genomic loci implicated in the carotenoid biosynthesis.

Of note, JoinTRV allowed CRISPR/Cas9-mediated heritable plant genome editing and tissue culture-free rescue of mutant progeny. Mutations at targeted loci were identified in more than half of the seedlings originating from Cas9-transgenic plants inoculated with a JoinTRV system that expressed sgRNAs fused to cell-to-cell mobile sequences.[15]

Novel methodological concepts for biosystems engineering are necessary to meet the ever-increasing human needs. Our pLX vectors and JoinTRV with proven flexibility and robustness will facilitate engineering and prototyping of next-generation viral vectors for crop trait reprograming, *ad hoc* plant genomic manipulation or new product development.

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REFERENCES

(1) Gleba, Y. Y.; Tusé, D.; Giritch, A. *Curr. Top. Microbiol. Immunol.* **2014**, *375*, 155–192. https://doi.org/10.1007/82_2013_352.
(2) Pasin, F.; Menzel, W.; Daròs, J.-A. *Plant Biotechnol. J.* **2019**, *17* (6), 1010–1026. https://doi.org/10.1111/pbi.13084.
(3) Torti, S.; Schlesier, R.; Thümmler, A.; Bartels, D.; Römer, P.; Koch, B.; Werner, S.; Panwar, V.; Kanyuka, K.; Wirén, N. von; Jones, J. D. G.; Hause, G.; Giritch, A.; Gleba, Y. *Nat. Plants* **2021**, *7* (2), 159–171. https://doi.org/10.1038/s41477-021-00851-y.
(4) Khakhar, A.; Voytas, D. F. *Front. Plant Sci.* **2021**, *12*, 668580. https://doi.org/10.3389/fpls.2021.668580.
(5) Oh, Y.; Kim, H.; Kim, S.-G. *Curr. Opin. Plant Biol.* **2021**, *60*, 101992. https://doi.org/10.1016/j.pbi.2020.101992.
(6) Ali, Z.; Abul-faraj, A.; Li, L.; Ghosh, N.; Piatek, M.; Mahjoub, A.; Aouida, M.; Piatek, A.; Baltes, N. J.; Voytas, D. F.; Dinesh-Kumar, S.; Mahfouz, M. M. *Mol. Plant* **2015**, *8* (8), 1288–1291. https://doi.org/10.1016/j.molp.2015.02.011.
(7) Ellison, E. E.; Nagalakshmi, U.; Gamo, M. E.; Huang, P.-J.; Dinesh-Kumar, S.; Voytas, D. F. *Nat. Plants* **2020**, *6* (6), 620–624. https://doi.org/10.1038/s41477-020-0670-y.
(8) Ma, X.; Zhang, X.; Liu, H.; Li, Z. *Nat. Plants* **2020**, *6* (7), 773–779. https://doi.org/10.1038/s41477-020-0704-5.
(9) Ariga, H.; Toki, S.; Ishibashi, K. *Plant Cell Physiol.* **2020**, *61* (11), 1946–1953. https://doi.org/10.1093/pcp/pcaa123.
(10) Uranga, M.; Vazquez-Vilar, M.; Orzáez, D.; Daròs, J.-A. *CRISPR J.* **2021**, *4* (5), 761–769. https://doi.org/10.1089/crispr.2021.0049.
(11) Uranga, M.; Aragonés, V.; Selma, S.; Vázquez-Vilar, M.; Orzáez, D.; Daròs, J.-A. *Plant J.* **2021**, *106* (2), 555–565. https://doi.org/10.1111/tpj.15164.
(12) Pasin, F.; Bedoya, L. C.; Bernabé-Orts, J. M.; Gallo, A.; Simón-Mateo, C.; Orzaez, D.; García, J. A. *ACS Synth. Biol.* **2017**, *6* (10), 1962–1968. https://doi.org/10.1021/acssynbio.6b00354.
(13) Pasin, F.; Tseng, X.-A.; Bedoya, L. C.; Heydarnejad, J.; Deng, T.-C.; García, J. A.; Chen, Y.-R. *J. Virol. Methods* **2018**, *262*, 48–55. https://doi.org/10.1016/j.jviromet.2018.09.007.
(14) Pasin, F. *Biotechnol. J.* **2021**, *16* (5), e2000354. https://doi.org/10.1002/biot.202000354.
(15) Aragonés, V.; Aliaga, F.; Pasin, F.; Daròs, J.-A. *Simplifying Plant Gene Silencing and Genome Editing Logistics by a One-*Agrobacterium *System for Simultaneous Delivery of Multipartite Virus Vectors*; preprint; 2021. https://doi.org/10.22541/au.163309949.93160648/v1.