

SHORT COMMUNICATION OPEN ACCESS

Electrical Current-Mediated Transformation for Efficient Plant Genome Editing: A Case Study in Faba Bean

Sruthy Maria Augustine¹  | Anoop V. Cherian^{2,3} | Paridhi Paridhi¹ | Samson Ugwuanyi¹  | Md Mamunur Rashid¹ | Babette Knoblauch¹ | Stavros Tzigos¹ | Soni Savai Pullamsetti^{2,3,4} | Rod J. Snowdon¹ 

¹Department of Plant Breeding, IFZ Research Center for Biosystems, Land Use and Nutrition, Justus Liebig University, Giessen, Germany | ²Department of Internal Medicine, Universities of Giessen and Marburg Lung Center (UGMLC), Member of the German Center for Lung Research (DZL), Excellence Cluster Cardio-Pulmonary Institute (CPI), Justus Liebig University, Giessen, Germany | ³Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany | ⁴Institute for Lung Health (ILH), Justus Liebig University, Giessen, Germany

Correspondence: Sruthy Maria Augustine (sruthy.augustine@agr.uni-giessen.de)

Received: 12 December 2024 | **Revised:** 14 April 2025 | **Accepted:** 15 April 2025

Funding: This work was supported by DFG grant 469336000 to R.J.S. from the German Research Society (DFG) for the International Research Training Group IRTG 2843 Accelerating Crop Genetic Gain.

Genome editing technologies have great potential to accelerate plant breeding, but delivery of editing constructs is difficult in many crop species because they are recalcitrant to transformation or tissue culture. Here, we present a transformative method for delivering ribonucleoprotein (RNP) complexes and plasmid vectors to intact, regenerable plant tissues. This is the initial description and proof of concept for a novel transformation method for genome editing in faba beans, exemplifying a tissue culture recalcitrant crop species. This is achieved by applying an electric current to make plant cell walls and membranes permeable, facilitating the entry of macromolecular constructs into the plant cell and nucleus (Furuhata et al. 2019). This study presents a genome editing method applied to faba bean (*Vicia faba* L.), an early domesticated crop and an important cool-season legume in global agriculture, recognised for its nitrogen-fixing abilities and as a key protein source in numerous countries (Jithesh et al. 2024; Jayakodi et al. 2023). A major limitation in faba bean research to date has been the absence of a reliable transformation or genome-editing methods.

To prepare, the excised seed embryo (Figure S3) is perforated five to eight times with a 26-gauge needle. A droplet of liquid containing the desired macromolecular construct, such as an RNP complex or plasmid, is then applied to the perforated surface. Two 26-gauge needles are then inserted into the plant tissue, and transfection is achieved by applying an electric current via a 24–28 V

battery (Figure 1A, Appendix S1). Details about the electrode assembly and safe use are provided in Appendix S2. To evaluate the effectiveness of electric current-mediated transfection in faba bean, we introduced the green fluorescent protein (GFP)-expressing transgene construct pLH-6000-GFP (250 ng/μL) (Figure S1; Imani et al. 2011) into faba bean embryos using this technique. Embryos were extracted from mature seeds that had been soaked in sterile MilliQ water for around 16 h. Viable embryos were retrieved and transferred to a standard 100x15 mm petri dish. The needles are connected to the battery prior to insertion into the embryo. One needle was inserted 2–3 mm into the embryo, while the second needle was briefly touched onto the first one for less than one second, creating an electric current to destabilize the plant cell membranes and facilitate plasmid transfection into cells and nuclei. The pulsing was repeated 3–4 times within 10–15 s.

GFP presence was confirmed two days after application of the electric current using confocal microscopy (Figure 1B, Video 1), with fluorescence detected using a 488 nm laser for excitation and an emission peak at 509–510 nm. Post-transfection, embryos expressing GFP were cultured on MS medium (4.4 g/L MS salts with vitamins, 20 g/L sucrose, 7 g/L agar, pH 5.8) supplemented with 1.5 mg/L IAA and maintained at 20°C–23°C with a 16-h photoperiod (7000 lx). The transformed tissue was subcultured every 10–12 days until shoot emergence (Figure S5). The plants were then transferred to greenhouse conditions alongside wild-type

Sruthy Maria Augustine and Anoop V. Cherian have equal contributions.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *Legume Science* published by Wiley Periodicals LLC.

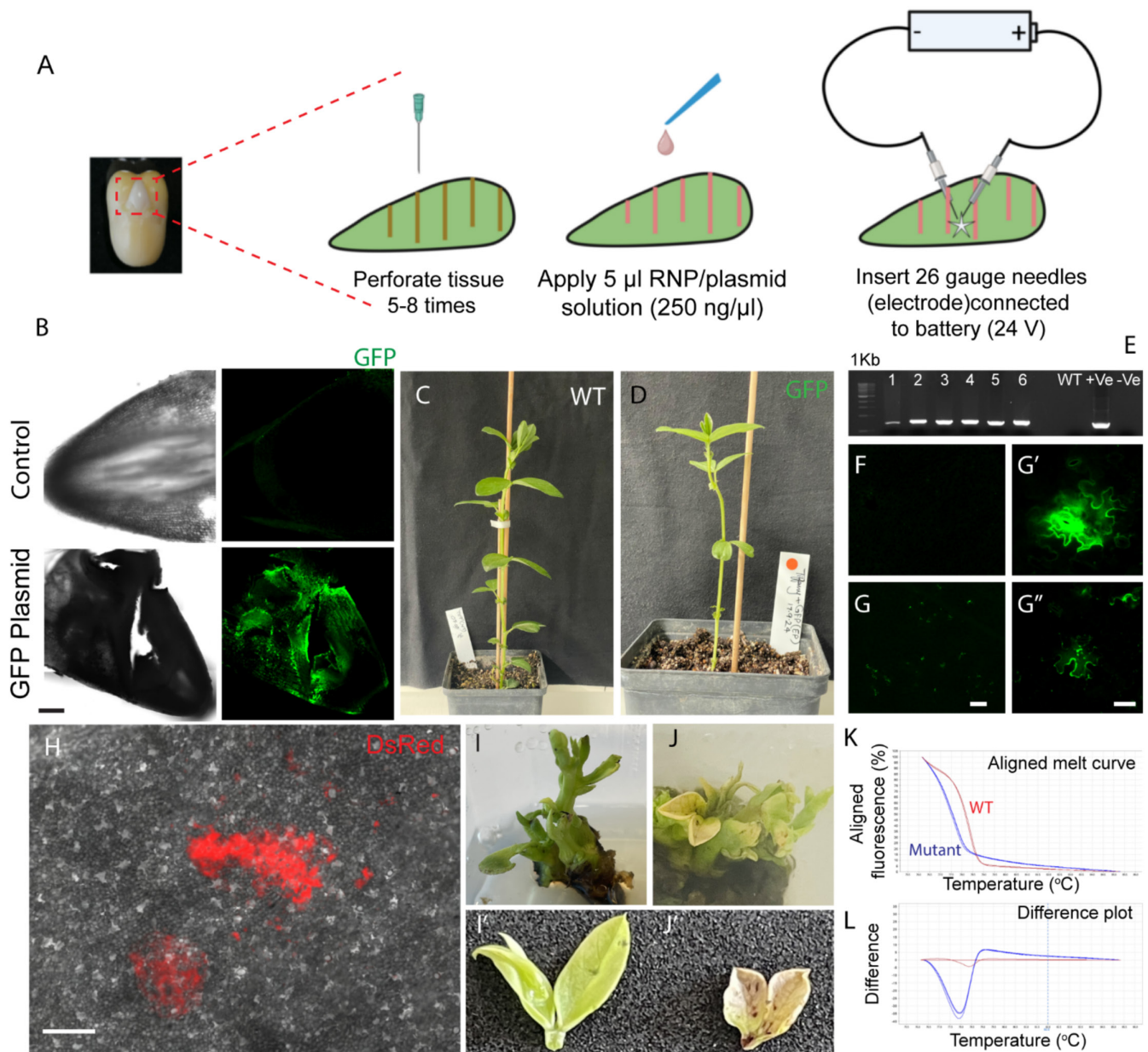


FIGURE 1 | Electric current mediated genome editing in faba bean (A) Electric current-mediated transfection of an intact plant embryo via battery-connected needle electrodes. Created with the help of [BioRender.com](#). (B) Verification of electric current-mediated transformation of *V. faba* embryos with the pLH-6000-GFP construct by evaluation of GFP expression in the wild type control (above) and transfected embryo (below) 2 days after transfection. Scale bar = 500 μ m. (C) Regenerated wild type control. (D) Regenerated GFP overexpressed faba bean. (E) PCR confirmation of the hpt gene in the GFP overexpressed faba bean plants. Lane 1 Kb: 1 Kb DNA marker; lanes 1–6: 735 bp amplicon of hpt gene from individual events; lane WT: untransformed control; lane (+ve): positive control; lane (–ve): negative control. (F) GFP analysis in wild type control. (G) GFP expression in leaf tissue of the GFP overexpressed plant. Scale bar = 50 μ m. (G', G'') Zoomed regions of the GFP overexpressed cells. Scale bar = 50 μ m. (H) DsRed fluorescence detection on the faba bean leaf tissue after electric current mediated transfection. Scale bar = 500 μ m. (I, I') Wild type faba bean plants. (J, J') Chimeric albino mutant plants resulting in mutation of the *pds* gene. High-resolution melt analysis of mutant plants generated through gene editing by electric current-mediated transfection resulting in mutation of the *pds* gene (K) Aligned melt curve (L) Difference plot.

controls (Figure 1C and 1D). Genomic DNA was extracted at the 4–6 leaf stage following Doyle and Doyle (1990), and the presence of the hygromycin (*hpt*) marker gene was confirmed via PCR (Figure 1E). The transgenic status was further validated by observing GFP expression in leaves with confocal microscopy (Figure 1F, G, G', G''). We acquired 11 GFP-overexpressing plants from 57 embryos transformed via electric current mediated transformation, representing an efficiency of 19%.

In addition to embryos, we assessed the efficacy of this method for introducing constructs and RNPs into leaf tissues using 3–4-month-old faba bean leaves. The technique successfully introduced a red fluorescent protein (DsRed) R2G mutant from pGJ1425 (MPI, Cologne, Germany) (Sack et al. 2015) into leaf tissue, indicating that this method is adaptable to both embryos and leaf tissues as explants (Figure 1H). Details regarding the preparation and imaging of leaf tissue are provided in the Appendix S3.

To demonstrate DNA-free genome editing, we targeted the endogenous phytoene desaturase gene (*PDS*), where mutations yield a visually identifiable albino phenotype, providing an efficient measure of mutation success. The gene structure is shown in Figure S2. The gene is identified as Vfaba.Tiffany.R1.2g090080, and the corresponding transcript is Vfaba.Tiffany.R1.2g090080.1 (Tiffany sequence available at <https://projects.au.dk/fabagenome/genomics-data>; Jayakodi et al. 2023). Target regions within the *PDS* gene were amplified from genomic DNA using Q5 high-fidelity DNA polymerase, purified with the QIAquick gel extraction kit (QIAGEN, Hilden, Germany), and sequenced using Sanger sequencing before crRNA design, as specified in Table S1. crRNA sequences were generated using the online tool “CRISPRdirect” (<https://crispr.dbcls.jp/>). The crRNA targeting the *PDS* gene was 5'-GAACCATGGTCTCGTTTGA-3'.

RNP complexes were prepared with crRNA, tracrRNA, and Cas9 protein (synthesized by Integrated DNA Technologies, Inc., Iowa, USA). For all experiments, chemically modified crRNA-XT was used to enhance stability and performance. Equimolar crRNA and tracrRNA were mixed to a final concentration of 100 μ M and heated at 95°C for 5 min. The RNP was produced using a 1:1.2 ratio of Cas9 to gRNA. 120 pmol of gRNA mix, 104 pmol of Cas9 protein, and 2.1 μ L of phosphate-buffered saline (PBS) were combined for a total RNP volume of 5 μ L and incubated at room temperature for 20 min. The RNP complex was then immediately administered to freshly isolated embryos, eliminating the need for labour-intensive protoplast or zygote preparations. Following RNP delivery, embryos were cultured on MS medium at 20°C–23°C without selection reagents. Chimeric albino mutants from *PDS* gene knockouts were identified visually (Figure 1J, J', S4), with wild-type controls shown in Figure I and I'. Following regeneration, high-resolution melting analysis (HRMA), a PCR-based method, was used to detect chimeric and heterozygous mutants (Denbow et al. 2018; Li et al. 2018; Thomas et al. 2014). In the analysis of 22 plants from the *PDS* gene editing experiment, 11 exhibited mutations at the T0 stage. Figure 1K,L shows the melting curves of *PDS* mutants compared to wild-type controls, with additional HRMA curve images provided in Figure S6. The amplicon measured 317 bp in length, with conditions and primers detailed in Table S2.

Our electric current-mediated transformation achieved a 50% mutation efficiency in the *PDS* gene within approximately 7–8 months. The findings indicate that the electric current-mediated transformation method may be effective for genome editing in recalcitrant species such as faba bean. Direct transformation or mutation of intact embryos can overcome difficulties in large-seeded plant species, which are recalcitrant to tissue culture regeneration from alternative explants. This straightforward, cost-effective method, which requires minimal technical training, is applicable to both leaf and embryo tissues, making it broadly useful for crop improvement. This technique holds promise for developing new crop varieties that can better respond to global climate challenges.

Author Contributions

S.M.A. and A.V.C. designed the study, conducted experiments and data analyses, and wrote the manuscript. P.P., B.K., and S.T. conducted experiments, maintained all the plants and provided technical assistance

in the work. S.U. provided the bioinformatics support in the study. M.M.R. supported in genomic DNA extraction. S.S.P. provided the confocal microscope facility, and R.J.S. conceptualised and critically reviewed the manuscript. All authors have reviewed and approved to the final manuscript.

Acknowledgements

This work was supported by DFG grant 469336000 to RJS from the German Research Society (DFG) for the International Research Training Group IRTG 2843 Accelerating Crop Genetic Gain. The authors thank Norddeutsche Pflanzenzucht Hans-Georg Lembke KG (NPZ, Hohenlieth, Germany) for providing the faba bean seeds used in the study. Open Access funding enabled and organized by Projekt DEAL

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

References

- Denbow, C., S. C. Ehivet, and S. Okumoto. 2018. “High Resolution Melting Temperature Analysis to Identify CRISPR/Cas9 Mutants From Arabidopsis.” *Bio-Protocol* 8: e2944.
- Doyle, J. J., and J. L. Doyle. 1990. “Isolation of Plant DNA From Fresh Tissue.” *Focus* 12, no. 1: 13–15.
- Furuhata, Y., A. Sakai, T. Murakami, et al. 2019. “A Method Using Electroporation for the Protein Delivery of Cre Recombinase Into Cultured Arabidopsis Cells With an Intact Cell Wall.” *Scientific Reports* 9: 2163.
- Imani, J., L. Li, P. Schäfer, and K. H. Kogel. 2011. “STARTS – A Stable Root Transformation System for Rapid Functional Analyses of Proteins of the Monocot Model Plant Barley.” *TPJ* 67, no. 4: 726–735.
- Jayakodi, M., A. A. Golick, J. Kreplak, et al. 2023. “The Giant Diploid Faba Genome Unlocks Variation in a Global Protein Crop.” *Nature* 615: 652–659. <https://doi.org/10.1038/s41586-023-05791-5>.
- Jithesh, T., E. K. James, P. P. M. Iannetta, B. Howard, E. Dickin, and J. M. Monaghan. 2024. “Recent Progress and Potential Future Directions to Enhance Biological Nitrogen Fixation in Faba Bean (*Vicia faba* L.).” *PEI Journal* 5, no. 3: e10145. <https://doi.org/10.1002/pei3.10145>.
- Li, S., S. Liu, Y. Liu, et al. 2018. “HRM-Facilitated Rapid Identification and Genotyping of Mutations Induced by CRISPR/Cas9 Mutagenesis in Rice.” *Crop Breeding and Applied Biotechnology* 18: 184–191.
- Sack, M., T. Rademacher, H. Spiegel, et al. 2015. “From Gene to Harvest: Insights Into Upstream Process Development for the GMP Production of a Monoclonal Antibody in Transgenic Tobacco Plants.” *Plant Biotechnology Journal* 13: 1094–1105.
- Thomas, H. R., S. M. Percival, B. K. Yoder, and J. M. Parant. 2014. “High-Throughput Genomeediting and Phenotyping Facilitated by Highresolution Melting Curve Analysis.” *PLoS ONE* 9, no. 12: e114632.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.