

# Agrobacterium-Mediated Transient Gene Expression Optimized for the Bioenergy Crop *Camelina sativa*

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## Abstract

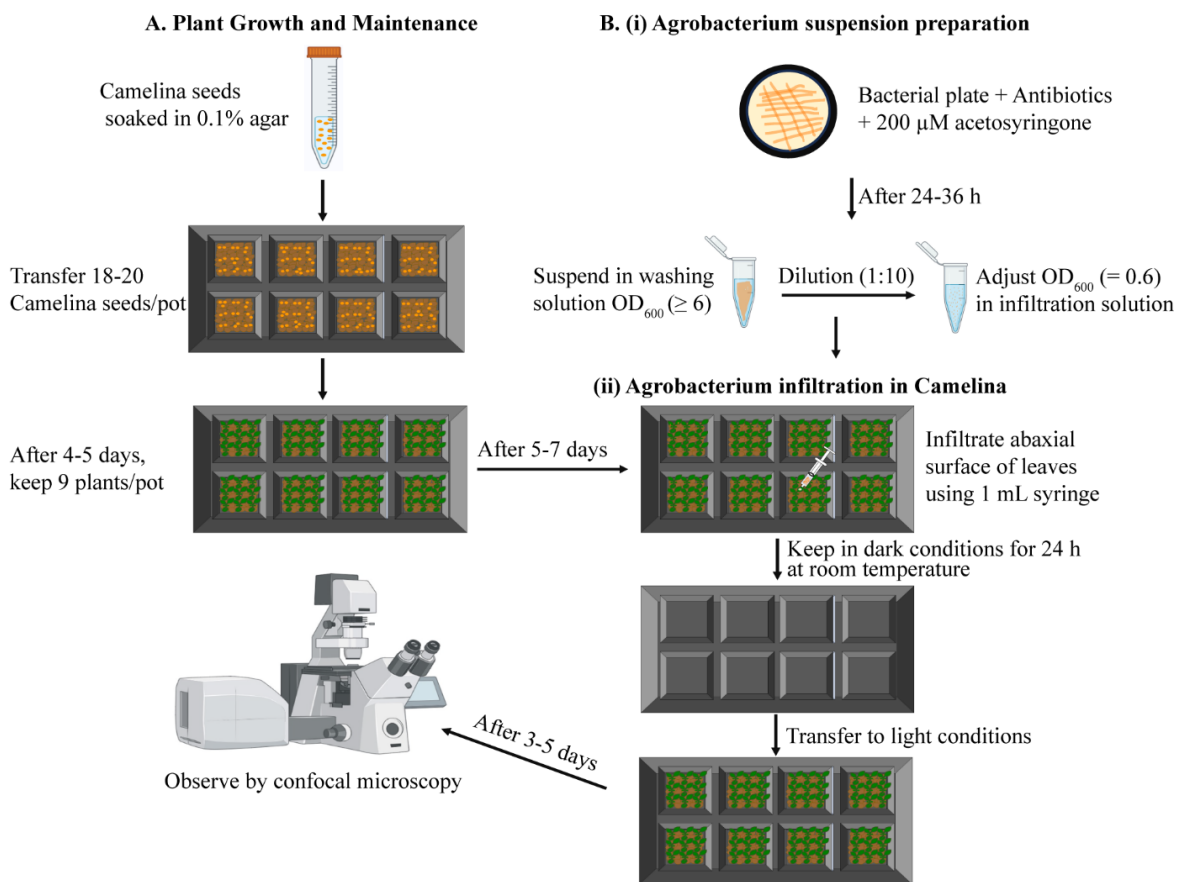
*Camelina sativa*, a Brassicaceae family crop, is used for fodder, human food, and biofuels. Its relatively high resistance to abiotic and biotic stresses, as well as being a climate-resilient oilseed crop, has contributed to its popularity. *Camelina's* seed yield and oil contents have been improved using various technologies like RNAi and CRISPR/Cas9 genome editing. A stable transformation system for protein localization and other cell autonomous investigations, on the other hand, is tedious and time consuming. This study describes a transient gene expression protocol for *Camelina sativa* cultivar DH55 leaves using *Agrobacterium* strain C58C1. The method is suitable for subcellular protein localization and colocalization studies and can be used with both constitutive and chemically induced genes. We report the subcellular localization of the N-terminal ER membrane signal anchor region (1–32 aa) of the *At3G28580* gene-encoded protein from *Arabidopsis* in intact leaves and the expression and localization of other known organelle markers. This method offers a fast and convenient way to study proteins in the commercially important *Camelina* crop system.

## Key features

- This method is based on the approach of Zhang et al. [1] and has been optimized for bioenergy crop *Camelina* species.
- A constitutive and inducible transient gene expression in the hexaploid species *Camelina sativa* cultivar DH55.
- Requires only 16–18 days to complete with high efficacy.

**Keywords:** *Camelina sativa*, Transient gene expression, Brassicaceae, Subcellular organelle localization, *Agrobacterium*

## Graphical overview



### *Agrobacterium*-mediated transient gene expression optimized for *Camelina sativa*

## Background

*Camelina sativa*, an important Brassicaceae family crop and crop model, is used to make fodder, human food, industrial chemical raw materials, biofuels, and compost to enhance soil properties [2]. It is planted worldwide, particularly in North America, Europe, and Asia, and is also known as large-seeded False Flax seed due to its high level of polyunsaturated fatty acids [3]. *Camelina* has lately become noted for its potential to be a high-value, climate-resilient oilseed crop owing to its resistance to a variety of abiotic and biotic stresses [4].

Using different technologies like classical breeding, transgenics, and CRISPR/Cas9 genome editing, various genetic improvements in *Camelina* have been made to increase its seed yield and oil contents and reduce its anti-nutritional glucosinolate content. Stable transformation systems that employ the floral-dip method and in vitro leaf explants are available in *Camelina sativa* [5]. Nevertheless, the transformation efficiency is very low (i.e., 0.8%–1%) and the method is laborious [6,7]. While an *Agrobacterium*-mediated infiltration system for rapid and transient gene expression for studying protein localization, protein–protein interaction, etc., has been well established in *Nicotiana benthamiana*, this technique has had limited applications for other crop species [8]. Hence, with regards to time and labor efficiency, the development of a transient expression system will provide an alternative and convenient way to study genes in *Camelina*.

Previously, Zhang et al. [1] developed a transient gene expression protocol using different strains of *Agrobacterium* in the model plant *Arabidopsis thaliana*, along with seven other plant species: *Brassica oleracea*, *Capsella rubella*, *Thellungiella salsuginea*, *T. halophila*, *Solanum tuberosum*, *Capsicum annum*, and *N. benthamiana*. Herein, we

modified this protocol for *Camelina sativa* cultivar DH55. The major modification is the age of the plants, which is younger than that described by Zhang et al. [1], enabling more rapid acquisition of data. In addition, this optimized protocol functions with constitutive and inducible promoters and is suitable for both subcellular localization and colocalization of proteins in *Camelina*. Using the leaves of 10–12-days-old plants infected with *Agrobacterium* strain C58C1, we show the subcellular localization of the organelle markers to chloroplasts, peroxisomes, Golgi bodies, and ER [9]. Leaves of 14-day-old plants showed a significantly decreased transformation efficiency, highlighting the benefit of using younger plants for these experiments. We also report the intracellular localization of the N-terminal ER signal (1–32 aa) region of the protein encoded by the *At3G28580* gene in intact leaves. This study demonstrates an efficient transient gene expression protocol for *Camelina*.

## Materials and reagents

### Biological materials

1. *Camelina sativa* cultivar DH55 seeds
2. *Agrobacterium* strain C58C1 containing constitutive or chemically inducible plasmid expression constructs. Plasmids used in this study harbor constructs encoding chloroplast-GFP (CD3-995), peroxisomes-mCherry (CD3-983), Golgi body-mCherry (CD3-967), ER-mCherry (CD3-959) as described in Nelson et al. [9], and Dex-inducible pBAV150 [10] plasmid with DNA encoding the N-terminal (1–32 aa) region of a protein coded by the *At3G28580* gene

### Reagents

1. Plastic domes (Hummert International, catalog number: 11-33480) and trays (Hummert International, catalog number: 11-33010)
2. Pots 8 cells; width × length × depth: 12.34 × 12.34 × 5.77 cm (T.O. PLASTICS, catalog number: 715352C)
3. Soil (Berger 60, catalog number: BM2; BM6, mixed in 1:1 ratio)
4. Yeast extract (Fisher Scientific, catalog number: BP1422-500)
5. Bacto™ peptone (Thermo Fisher Scientific, catalog number: 211677)
6. Sucrose (Fisher Scientific, catalog number: S5-500)
7. MgSO<sub>4</sub>·7H<sub>2</sub>O (Fisher Scientific, catalog number: 10034-99-8)
8. NH<sub>4</sub>Cl (Sigma, catalog number: 12125-02-9)
9. KCl (Fisher Scientific, catalog number: P217-500)
10. CaCl<sub>2</sub> (Sigma, catalog number: C2661)
11. FeSO<sub>4</sub>·7H<sub>2</sub>O (Sigma, catalog number: F-7002)
12. NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (JT Baker, catalog number: JTB-3818-05)
13. Na<sub>2</sub>HPO<sub>4</sub> (Fisher Scientific, catalog number: 7558-79-4)
14. Glucose (Sigma, catalog number: G7021)
15. MES (Sigma, catalog number: M8250)
16. MgCl<sub>2</sub>·6H<sub>2</sub>O (Fisher Scientific, catalog number: BP214-500)
17. Murashige and Skoog medium (Sigma, catalog number: M5519)
18. Bleach (Clorox Concentrated Germicidal Bleach)
19. Ethanol (Decon Labs Inc., catalog number: 2701)
20. Agarose (GOLDBIO, catalog number: 9012-36-6)
21. Silwet L-77 (Vac-In-Stuff, catalog number: VIS-30)
22. Tween® 20 (Hofer, catalog number: 9005-64-5, GR128-500)
23. Antibiotics: kanamycin sulfate (Kan) and rifampicin (Rif) (Fisher Scientific, catalog number: BP906-5, 13292-46-1, respectively)
24. Nalgene™ Rapid-Flow™ sterile disposable filter units (Thermo Fisher Scientific, catalog number: 568-0020)
25. Dexamethasone (Sigma, catalog number: D1756)

26. Perfluorodecalin (Strem Chemicals, catalog number: 09-5960)
27. Dimethyl sulfoxide (DMSO) (Sigma, catalog number: D8418)
28. Agar (Fisher Scientific, catalog number: BP1423-500)
29. Acetosyringone (Sigma, catalog number: D134406)

## Solutions

1. Washing solution (see Recipes)
2. Infiltration solution (see Recipes)
3. *Agrobacterium* growth media (see Recipes)
4. 30  $\mu$ M Dexamethasone (Dex) solution with 0.04% Tween-20 (see Recipes)
5. 70% ethanol (see Recipes)
6. 0.1% agar media (see Recipes)
7. Phosphate buffer (50 mM, 25 $\times$ , pH 5.5) (see Recipes)

## Recipes

Recipes 1, 2, and 3 are adapted from Zhang et al. [1].

### 1. Washing solution (1 L)

Reagent	Final Concentration	Volume
MgCl <sub>2</sub> (1 M)	10 mM	10 mL
Acetosyringone (0.1 M)	100 $\mu$ M	1 mL
ddH <sub>2</sub> O	n/a	989 mL
Total	n/a	1 L

### 2. Infiltration solution (1 L)

*Note: Water should be added in increments of 100 mL. Allow the solution to mix completely before adding any more water. Murashige and Skoog medium and sucrose will take a substantial volume when fully dissolved. Adjust pH to 6.0 using 1 M KOH; use freshly prepared.*

Reagent	Final Concentration	Quantity or Volume
Murashige and Skoog medium	1/4 $\times$ (w/v)	1.1 g
Sucrose	1% (w/v)	10 g
Acetosyringone (0.1 M)	100 $\mu$ M	1 mL
Silwet L-77	0.01% (v/v)	100 $\mu$ L
ddH <sub>2</sub> O	n/a	see note
Total	n/a	1 L

### 3. *Agrobacterium* growth media (1 L)

*Note: Adjust the pH to 5.5 using 1 M KOH and autoclave the media. After autoclaving, add the filtered induce buffer components. Acetosyringone is prepared in DMSO.*

Reagent	Final Concentration	Quantity or Volume
Yeast extract	0.1% (w/v)	1.0 g
Bacto™ peptone	0.5% (w/v)	5.0 g
Sucrose	0.5% (w/v)	5.0 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	2.03 mM	0.5 g
Agarose	1% (w/v)	10.0 g
NH <sub>4</sub> Cl	18.70 mM	1.0 g

KCl	2.01 mM	0.15 g
CaCl <sub>2</sub>	90.10 μM	0.01 g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	8.99 μM	0.0025 g
ddH <sub>2</sub> O	n/a	889 mL (see note)

**Induce buffer composition:**

Phosphate buffer (50 mM, 25×, pH 5.5)	1×	40 mL
Glucose (20%, 20×)	1×	50 mL
MES (1 M, 50×, pH 5.5)	1×	20 mL
Acetosyringone (200 mM, 1,000×)	1×	1 mL
Total	n/a	1 L

**4. 30 μM Dexamethasone (Dex) solution with 0.04% Tween-20 (100 mL)**

*Note: Dex is prepared in DMSO.*

Reagent	Final Concentration	Volume
Dex (30 mM)	30 μM	100 μL (see note)
Tween-20	0.04%	40 μL
ddH <sub>2</sub> O	n/a	99.86 mL
Total	n/a	100 mL

**5. 70% ethanol (100 mL)**

Reagent	Final Concentration	Volume
Ethanol (95%)	70%	73.7 mL
ddH <sub>2</sub> O	n/a	26.3 mL
Total	n/a	100 mL

**6. 0.1% agar media (100 mL)**

*Note: Autoclave the media and stir before it cools.*

Reagent	Final Concentration	Volume
Agar	0.1%	100 mg
ddH <sub>2</sub> O	n/a	100 mL
Total	n/a	100 mL (see note)

**7. Phosphate buffer (50 mM, 25×, pH 5.5; 100 mL)**

*Note: Adjust the pH to 5.5 using 1 M KOH.*

Reagent	Final Concentration	Volume
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	48.02 mM	662.7 mg
Na <sub>2</sub> HPO <sub>4</sub>	1.98 mM	28.05 mg
ddH <sub>2</sub> O	n/a	100 mL
Total	n/a	100 mL (see note)

**Laboratory supplies**

1. Laboratory glassware
2. Pipette tips: 10 μL, 200 μL, and 1,000 μL (USA Scientific, catalog number: 1111-3700, 1111-1700, 1112-1720)
3. Black marker (Sharpie Permanent Markers, catalog number: 33861PP)
4. Kimwipes (Fisher Scientific, catalog number: 06-666A)
5. 15 mL and 50 mL centrifuge tubes (Fisher Scientific, catalog numbers: 14-959-53A, 14-432-22)

6. 9 cm round Petri dishes (Fisher Scientific, catalog number: FB0875712)
7. 1.5 mL microcentrifuge tubes (Fisher Scientific, catalog number: 01-549-746)
8. 1 mL tuberculin syringes without needle (BD Biosciences, catalog number: 309659)
9. Damp paper towel (Georgia-Pacific, catalog number: P200)
10. Sterile double-distilled water
11. Micropipettes 20  $\mu$ L, 200  $\mu$ L, and 1 mL (Gilson, catalog number: F144056M, F144058M, F144059M, respectively)
12. 1 L beaker (Pyrex, catalog number: 1000)

## Equipment

1. Cork borer 4 mm diameter
2. Autoclave (Primus Sterilizer Co. Inc. 1317, catalog number: 09415.1256)
3. Fisher vortex Genie 2 (Fisher Scientific, catalog number: 12-812)
4. Plant growth chamber or walk-in growth room with humidity maintained at  $\geq 50\%$ . Light intensity should be  $135\text{--}145 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  at soil level
5. Freezer ( $-80 \text{ }^\circ\text{C}$ ) (Panasonic VIP Plus, model: MDF-V76VC-PA)
6. Balance (Mettler Toledo, model: PB1501)
7. Spectrophotometer (Bio-Mini, model: SHIMADZU)
8. Laminar flow hood (SterilGARD, model: 3 Advance)
9. Incubator at  $28 \text{ }^\circ\text{C}$  (VWR, model: 3020)
10. Analytical balance for weighing chemicals
11. pH meter (SevenCompact, serial number: B408309625)
12. Confocal microscope (Zeiss, model: LSM 800)

## Software and datasets

1. Fiji (ImageJ, version 2.15.0, 10/12/2023)
2. Photoshop (Adobe PS, 22.4.3, 07/19/2021)
3. Prism v10.1 (GraphPad, 07/01/2023)
4. BioRender.com online tool

## Procedure

### A. Plant growth and maintenance

1. Soak non-sterilized, dry *Camelina* seeds ( $\sim 100\text{--}150$ ) in 4–6 mL of 0.1% sterile agar in 15 mL falcon tubes and keep at  $4 \text{ }^\circ\text{C}$  in the dark for 3–7 days. The *Camelina* seeds utilized in this study were multiplied and bulked inside the greenhouse chamber.
2. Sow approximately 18–20 seeds in individual soil mix-containing pots ( $12.34 \times 12.34 \times 5.77$  cm) with the help of a Pasteur pipette and cover with a transparent plastic lid to maintain moisture. Keep pots in a plant growth chamber or growth room chamber under 12 h light and 12 h dark conditions at  $22 \pm 2 \text{ }^\circ\text{C}$  day/night temperature regime and light intensity of  $\sim 135\text{--}145 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ . We use a mix of 50/50 sodium and metal halide light (but other lights might be suitable) at 50%–70% relative humidity.
3. After 4–5 days, thin seedlings to nine plants per pot and remove the lid. Select the seedlings that look visibly similar and discard the rest (Figure S1).
4. Allow seedlings to establish for the next 5–7 days until the first true leaves are completely opened (Figure

1).

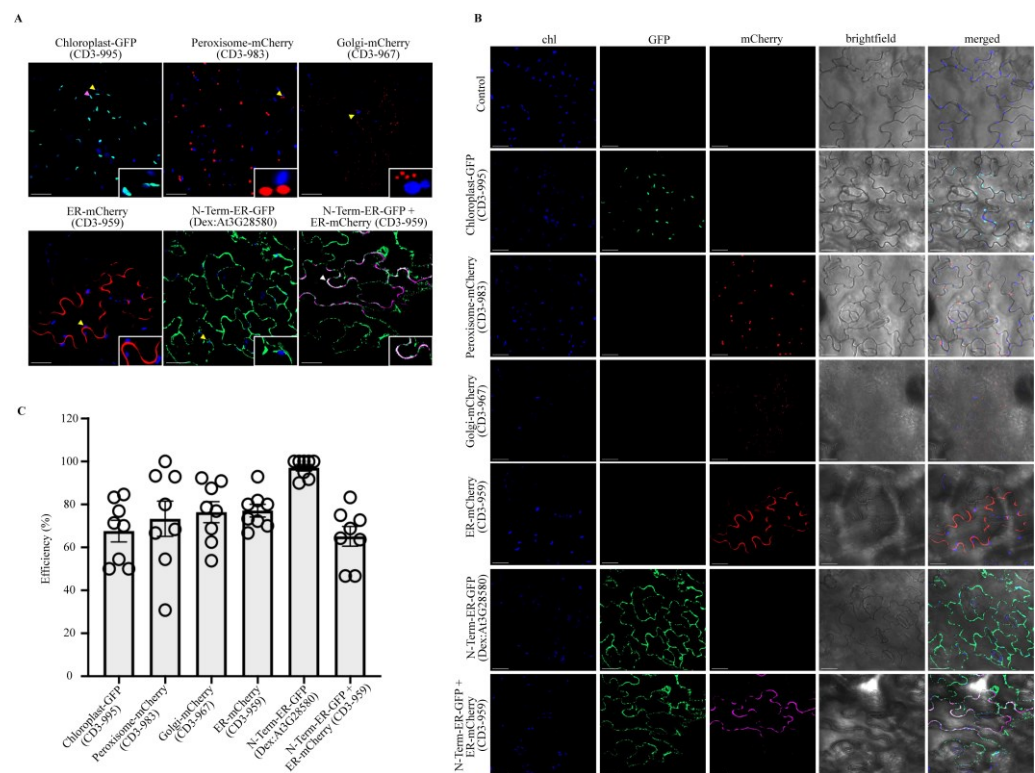


**Figure 1. Representative image depicting *Agrobacterium*-infiltrated plants of *Camelina sativa* DH55 cultivar.** Ten-to-twelve-day-old plants with opened true leaves were infiltrated with *Agrobacterium* strain C58C1 carrying desired constructs. White arrows indicate representative leaves imaged immediately after infiltration. The abaxial part of the leaves will have a wet appearance until the infused liquid containing *Agrobacterium* evaporates. Scale bar is in centimeters.

## B. *Agrobacterium* strain suspension preparation for infiltration

1. *Agrobacterium* suspension preparation
  - a. Streak an *Agrobacterium* strain C58C1 stock with the desired constructs on a sterile plate of *Agrobacterium* growth media containing appropriate antibiotics and 200  $\mu$ M acetosyringone inside a laminar airflow cabinet.  
Keep plates with bacteria at 28  $^{\circ}$ C for 24–36 h. In this work, marker plasmids of plastid-GFP (CD3-995), peroxisomes-mCherry (CD3-983), Golgi body-mCherry (CD3-967), ER-mCherry (CD3-959), and the N-terminal ER signal (1–32 aa) region of the At3G28580-encoded protein were used, and plates contained Rif (34 mg/mL) + Kan (50 mg/mL).
  - b. Scrape a thin layer of *Agrobacterium* cells from each plate using a 200  $\mu$ L pipette tip and resuspend bacteria in 1 mL of washing solution (see Recipes) in a 1.5 mL microcentrifuge tube by vortexing.
  - c. Dilute an aliquot of 100  $\mu$ L of resuspended *Agrobacterium* 10 $\times$  in order to measure the OD<sub>600</sub>. A value equal to or greater than 0.6 is desired in the final infiltration solution. Multiply this OD value by 10 to determine the OD<sub>600</sub> of the original undiluted resuspended *Agrobacterium*. This is done to obtain enough inoculum, sufficient to infiltrate all the true leaves per pot for each construct. After that, adjust the original suspension of *Agrobacterium* to an OD<sub>600</sub> of 0.6 using the infiltration solution (see Recipes). For colocalization studies, mix strains in a 1:1 ratio using an OD<sub>600</sub> of 0.6 for each strain and immediately infiltrate the leaves.
2. *Agrobacterium* infiltration in *Camelina*
  - a. Infiltrate the *Agrobacterium* in the infiltration solution into the abaxial surface of *Camelina* leaves (2 true leaves per plant) with the help of a 1 mL needleless plastic syringe. Use four or more plants per construct for infiltrations. Keep plants in the light for 1 h to allow the water-soaked leaves to dry before placing plants in the dark for 24 h at room temperature using plastic trays.
  - b. Return the *Agrobacterium*-infiltrated plants to a growth chamber or growth room for another 3–5 days before observing leaves using confocal microscopy. If using an inducible promoter, spray the inducer

over leaves 21 h before examination, as described in Banday et al. [11], using a confocal microscope (Figure 2A and 2B). In the example in Figure 2A, 30  $\mu$ M Dex with 0.04% Tween-20 was applied.



**Figure 2. *Agrobacterium*-mediated transient subcellular localization in *Camelina sativa* DH55 cultivar.** A. Upper panel: Confocal micrographs showing localization of Chloroplast-GFP (CD3-995), Peroxisome-mCherry (CD3-983), and Golgi-mCherry (CD3-967). Lower panel: Confocal micrographs of ER-mCherry (CD3-959), N-Term-ER-GFP (Dex: At3G28580; 1-32 aa region), and colocalization of N-Term-ER-GFP (Dex: At3G28580) with ER-mCherry (CD3-959; shown in magenta color). For localization and colocalization studies, images were taken 3 and 5 days after transfer to the light conditions, respectively. Chloroplast autofluorescence is shown in blue. Yellow arrowheads indicate chloroplast autofluorescence (shown in insets), magenta arrowheads indicate chloroplast-GFP overlap with chloroplast autofluorescence, and white arrows indicate colocalization of N-Term-ER-GFP (Dex: At3G28580) and ER-mCherry (CD3-959). Scale bars, 100  $\mu$ m. B. Details of the separate channels of individual confocal micrographs: chloroplast autofluorescence (Blue), GFP signal (Green), and mCherry signal (Red). For colocalization, the mCherry signal is shown in magenta color. Scale bars, 100  $\mu$ m. C. Efficiency of each transiently expressed construct. Data represent the average  $\pm$  SE (n = 8) in percentage (%) obtained from experiments performed on two different days. The four images from each independent experiment were used for the calculations. See Table S1 for details.

## Data analysis

The confocal microscope method was adapted from Banday et al. [11]. Zeiss LSM 800 was used to visualize GFP and mCherry fluorescence (Argon laser/excitation: 488 nm; emission collection: 505–530 nm for GFP; Argon laser/excitation: 594 nm; emission collection: 610–630 nm for mCherry) and chlorophyll autofluorescence (He-Ne laser/excitation: 633nm; emission collection: 650–750 nm or 645–700 nm). Images of abaxial leaf surface were captured using EC Plan-Neofluar 40/1.3 Oil DIC M27 objectives, pinhole at 1 AU for each channel, and



photomultipliers master gain between 450 and 700. The images were optical sections captured at  $1,024 \times 1,024$  pixels scanning resolution in maximum speed mode. Fluorescence of GFP, mCherry, and chlorophyll was acquired in sequential acquisition mode. Plant leaves were mounted in perfluorodecalin for optical enhancement [12]. ImageJ (Fiji) and Adobe PS were used to process the images. Each *C. sativa* imaging experiment included four independent samples per construct, and the experiments were done twice. Four images from different plant leaves from each experiment were used to calculate the efficiency of the transformation of cells per image. Using this protocol, all infiltrated leaves showed expression of the respective constructs. However, the efficiency of cells transformed/image was lower in the leaves of older plants (Table S1).

For calculation of efficiency analysis, the formula was adapted from Zhang et al. [1]. The graph was plotted using Prism software (Figure 2C and Table S1).

Efficiency (%) = Number of transiently transformed cells \*100/Total number of cells

## Validation of protocol

- This protocol was adapted from Zhang et al. [1] and optimized for the *Camelina* crop. The protocol was validated using five different constitutive or chemically inducible plasmid expression constructs.
- Each *Camelina* imaging experiment included four independent samples per construct, and the experiments were done twice. Four images from different plant leaves from each experiment were used to calculate the efficiency of the transformation of cells per image.
- The student's t-test shows that the efficiency of cells transformed/image was significantly lower in leaves of older plants (Table S1).

## General notes and troubleshooting

1. Plants' age is very important. Leaves of older *Camelina* plants require needle punctures for successful infiltration and the efficiency of transformation declines (Table S1).
2. Pots must be well watered during the experiment to maintain humidity.
3. Keep only nine plants in one pot; plant density affects the growth of plants. A higher number of plants results in slower growth. Conversely, a lower number of plants per pot hastens the growth of plants.
4. Do not keep bacterial cultures for more than 36 h.
5. For better infiltration, the infiltration solution should be introduced into the abaxial surfaces of leaves.
6. Take confocal images of the abaxial surface to obtain a greater number of cells in the same focal plane.
7. Use perfluorodecalin to prevent the leaves from drying and for better imaging.
8. Clean the workbench before making an Agrobacterial suspension with the help of 70% ethanol. Later on, after completion of infiltration in plants, the remaining bacterial suspension must be treated with bleach for 24 h before being discarded into the sink.

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## Competing interests

The authors declare that they have no conflicts of interest.

## References

1. Zhang, Y., Chen, M., Siemiatkowska, B., Toleco, M. R., Jing, Y., Strotmann, V., Zhang, J., Stahl, Y. and Fernie, A. R. (2020). A Highly Efficient Agrobacterium-Mediated Method for Transient Gene Expression and Functional Studies in Multiple Plant Species. *Plant Commun.* 1(5): 100028. <https://doi.org/10.1016/j.xplc.2020.100028>
2. Sydor, M., Kurasiak-Popowska, D., Stuper-Szablewska, K. and Rogoziński, T. (2022). *Camelina sativa*. Status quo and future perspectives. *Ind. Crops Prod.* 187: 115531. <https://doi.org/10.1016/j.indcrop.2022.115531>
3. Waraich, E. A., Ahmed, Z. I., Ahmad, R., Ashraf, M. Y., Saifullah, Naeem, M. and Rengel, Z. (2013). *Camelina sativa*, a climate proof crop, has high nutritive value and multiple uses: A review. *Aust. J. Crop Sci.* 7(10):1551–1559. [https://www.cropj.com/waraich\\_7\\_10\\_2013\\_1551\\_1559.pdf](https://www.cropj.com/waraich_7_10_2013_1551_1559.pdf)
4. Neupane, D., Lohaus, R. H., Solomon, J. K. Q. and Cushman, J. C. (2022). Realizing the Potential of *Camelina sativa* as a Bioenergy Crop for a Changing Global Climate. *Plants* 11(6): 772. <https://doi.org/10.3390/plants11060772>
5. Ghidoli, M., Ponzoni, E., Araniti, F., Miglio, D. and Pilu, R. (2023). Genetic Improvement of *Camelina sativa* (L.) Crantz: Opportunities and Challenges. *Plants* 12(3): 570. <https://doi.org/10.3390/plants12030570>
6. Lu, C. and Kang, J. (2007). Generation of transgenic plants of a potential oilseed crop *Camelina sativa* by Agrobacterium-mediated transformation. *Plant Cell Rep.* 27(2): 273–278. <https://doi.org/10.1007/s00299-007-0454-0>
7. Liu, X., Brost, J., Hutcheon, C., Guilfoil, R., Wilson, A. K., Leung, S., Shewmaker, C. K., Rooke, S., Nguyen, T., Kiser, J., et al. (2012). Transformation of the oilseed crop *Camelina sativa* by Agrobacterium-mediated floral dip and simple large-scale screening of transformants. *In Vitro Cell. Dev. Biol. Plant* 48(5): 462–468. <https://doi.org/10.1007/s11627-012-9459-7>
8. Krenek, P., Samajova, O., Luptovciak, I., Duskocilova, A., Komis, G. and Samaj, J. (2015). Transient plant transformation mediated by *Agrobacterium tumefaciens*: Principles, methods and applications. *Biotechnol. Adv.* 33(6): 1024–1042. <https://doi.org/10.1016/j.biotechadv.2015.03.012>
9. Nelson, B. K., Cai, X. and Nebenführ, A. (2007). A multicolored set of in vivo organelle markers for co-localization studies in *Arabidopsis* and other plants. *Plant J.* 51(6): 1126–1136. <https://doi.org/10.1111/j.1365-313x.2007.03212.x>
10. Vinatzer, B. A., Teitzel, G. M., Lee, M., Jelenska, J., Hotton, S., Fairfax, K., Jenrette, J. and Greenberg, J. T. (2006). The type III effector repertoire of *Pseudomonas syringae* pv. *syringae* B728a and its role in survival and disease on host and non-host plants. *Mol. Microbiol.* 62(1): 26–44. <https://doi.org/10.1111/j.1365-2958.2006.05350.x>
11. Banday, Z. Z., Cecchini, N. M., Speed, D. J., Scott, A. T., Parent, C., Hu, C. T., Filzen, R. C., Agbo, E. and Greenberg, J. T. (2022). Friend or foe: Hybrid proline-rich proteins determine how plants respond to beneficial and pathogenic microbes. *Plant Physiol.* 190(1): 860–881. <https://doi.org/10.1093/plphys/kiac263>
12. Littlejohn, G. R., Gouveia, J. D., Edner, C., Smirnoff, N. and Love, J. (2010). Perfluorodecalin enhances in vivo confocal microscopy resolution of *Arabidopsis thaliana* mesophyll. *New Phytol.* 186(4): 1018–1025. <https://doi.org/10.1111/j.1469-8137.2010.03244.x>

## Supplementary information

The following supporting information can be downloaded [here](#):

1. Figure S1: Visible appearance of 5-days-old seedling.
2. Table S1: Details of efficiency analysis in percentage.