obtain lines of «hairy roots» of P. peruviana and A. caudatus with selective genes: the phosphinothricin-N-acetyltransferase gene (bar), the neomycin phosphotransferase II (nptII) gene and the target gene of human interferon (ifn-a2b).

Agrobacterium rhizogenes-mediated transformation of *Amaranthus caudatus* cultivars and *Physalis peruviana*

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Introduction. *Agrobacterium tumefaciens*-mediated transformation is currently the most widely applied method for introducing foreign genes into plant cells, enabling both stable genomic integration and transient expression of transgenes. Transient expression assays offer particular advantages for rapid assessment of vector functionality, gene regulatory elements, and transformation efficiency prior to establishing stable transgenic lines, as foreign gene expression occurs within several days post-inoculation without requirement for T-DNA integration into the host genome . However, the effectiveness of *Agrobacterium*-mediated transformation varies considerably across plant species and even among genotypes within the same species, necessitating species-specific protocol optimization .

The RUBY reporter system, constructed on the basis of the betalain biosynthesis pathway, has emerged as a highly effective visual marker for monitoring genetic transformation and gene expression in plant tissues . Unlike classical reporters such as GUS, luciferase, or fluorescent proteins, RUBY drives the conversion of tyrosine to vividly red-pink betalain pigment, which is detectable by the naked eye without specialized equipment, chemical substrates, or destructive sample processing. The 35S::RUBY vector, driven by the constitutive CaMV 35S promoter, encodes three enzymes — CYP76AD1, DODA, and glucosyltransferase — organized as a single polyprotein linked by 2A peptides, making it a convenient tool for screening transformation events across diverse plant species .

Representatives of the family *Solanaceae* — including *Nicotiana*, *Petunia*, and *Physalis* — differ substantially in their susceptibility to *Agrobacterium*-mediated transformation. Therefore, comparative evaluation of *Agrobacterium*-mediated transformation efficiency across these species using a reliable visual reporter is of both methodological and practical significance.

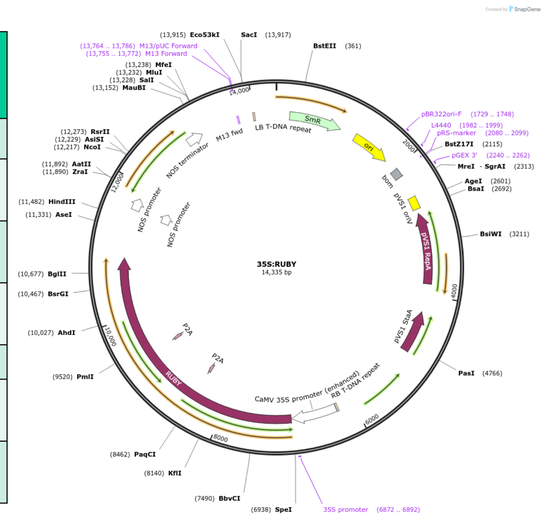
The aim of was to compare effectiveness of *Agrobacterium*-mediated transformation and evaluation of *Ruby* gene transient expression in tissues of *Physalis peruviana, P.ixocarpa* (variety Likhtaryk)*, P. pubescence* (variety Zharynka)*, Petunia hybrida M1, Petunia hybrida 5P, Nicotiana tabacum* cv. Wisconsin*, Nicotiana benthamiana*.

Materials and methods of investigation

Agrobacterium tumefaciens strain GV3101 carrying the 35S::Ruby genetic vector (He at al., 2020; Volmer et al., 2026) was grown in Luria-Bertani (LB) medium with antibiotics (50 mg/L rifampicin, 25 mg/L gentamicin, 50 mg/L spectinomycin) for 24 h with shaking. Agrobacterial suspension was centrifuged (5000 rpm, 12 min) and resuspended in ½ MS medium supplemented with 15 g/L sucrose (½ MS_{15}). Acetosyringone was added to a final concentration of 200 µM in 50 mL of *Agrobacterium* suspension.

Explants of *Physalis* species, *Petunia*, *N. tabacum*, and N. *benthamiana*, were co-cultivated with the *Agrobacterium* suspension for 30 min. Explants were then transferred to MS medium with 30 g/L sucrose (MS_{30}) without antibiotics for 24 h, followed by transfer to MS_{30} with 650 mg/L cefotaxime (to inhibit *Agrobacterium*) and 25 mg/L hygromycin (for plant selection). Petri dishes with control explants and with explants that were transformed were cultivated at 22–25°C, 3000–4500 lx, and a 14-h photoperiod. Transient *Ruby* expression was assessed visually; positive results were indicated by a color change from green to bright pink.

Plant species	Variety	Types of explants	Age of explants
Physalis peruviana		cotyledons	7-10-day old
P.ixocarpa	Likhtaryk.	cotyledons	7-10-day old
P. pubescence	Zharynka	cotyledons	7-10-day old
Petunia hybrida	M1	leaves	21-day old
Petunia hybrida	5P	leaves	21-day old
Nicotiana tabacum	Wisconsin	leaves	21-day old
Nicotiana benthamiana		leaves	21-day old



Results

Transient expression (pink color) was observed from the 3rd day; the maximum expression was observed on the 5th day after cocultivation of explants with *Agrobacterium*.

The efficiency of transformation was determined by calculating the percentage of explants with areas stained pink from the total number of explants used in each variant of the experiment. The efficiency of transformation for experimental plants presented in the table.

Species / Line	Variety / Line	Transient Expression (%)
Nicotiana tabacum	Wisconsin	100.0
Nicotiana benthamiana	—	56.4
Petunia hybrida	M1	79.0
Petunia hybrida	5P	26.3
Physalis peruviana	—	31.7
Physalis ixocarpa	Likhtaryk	8.6
Physalis pubescens	Zharynka	21.16

Conclusions

1. The 35S:RUBY vector is suitable for use in experiments aimed at obtaining transient expression of marker transgenes and genes of interest in transformed plants.
2. The efficiency of transformation was depended on genotypes, used types of explants and age of explants.
3. The highest efficiency of transformation in our experiments was detected for *N. tabacum*, *N. benthamiana*, *P. hybrida* M1 (100%, 56,4% and 79% respectively).

References

He Y, Zhang T, Sun H, Zhan H, Zhao Y. A reporter for noninvasively monitoring gene expression and plant transformation. Hortic Res. 2020 Sep 19;7(1):152. doi: 10.1038/s41438-020-00390-1. PMID: 33024566; PMCID: PMC7502077.

Vollmer, S. K., M. G.Stetter, and G.Hensel. 2026. “First Successful Targeted Mutagenesis Using CRISPR/Cas9 in Stably Transformed Grain Amaranth Tissue.” Plant Biotechnology Journal1–3. **https://doi.org/10.1111/pbi.70590**.

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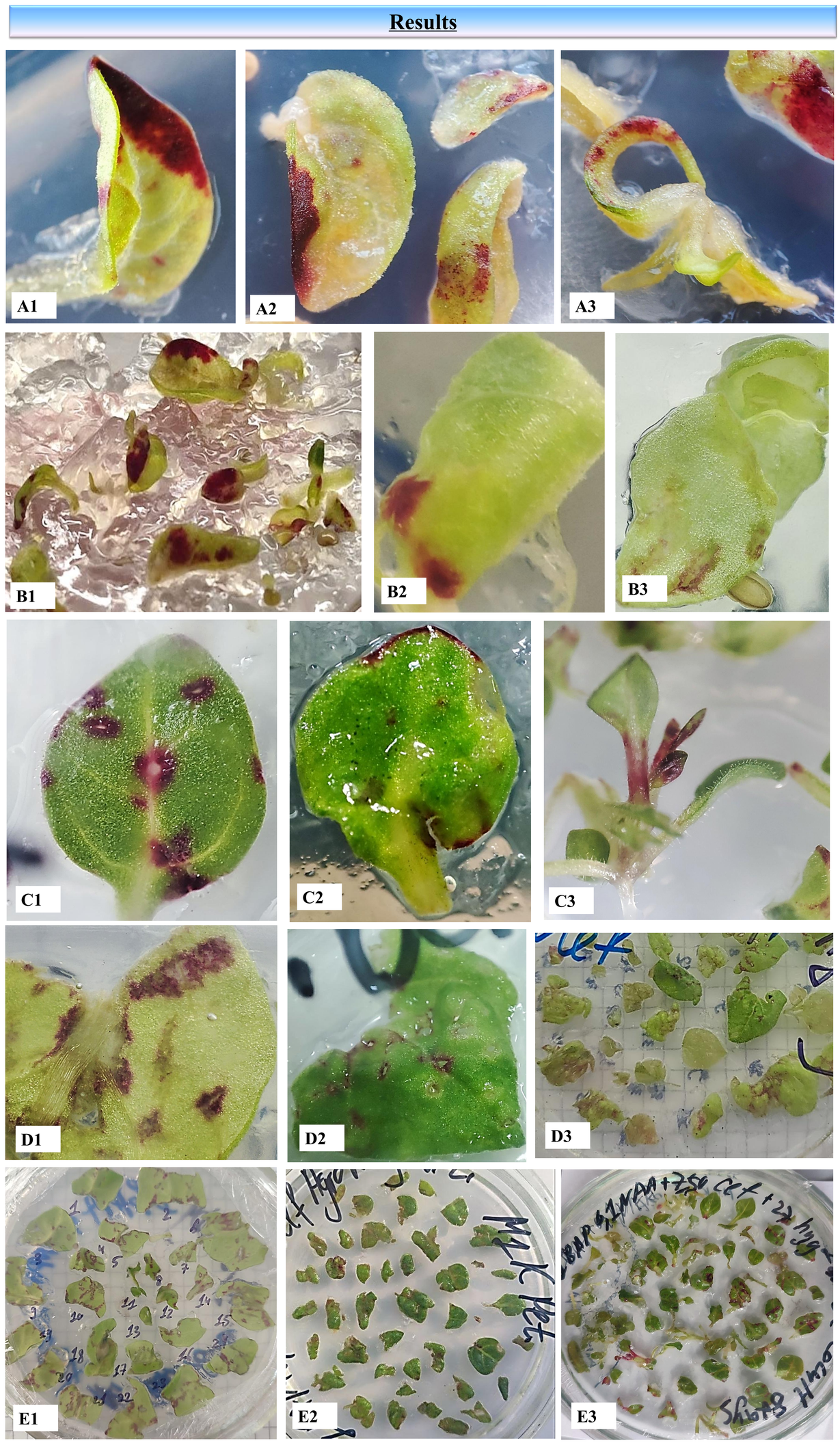
Results



Transient expression after 3rd day of experiment. A1-B1 – *P. peruviana;* B2 - *P. pubescence* (variety Zharynka); B3 - *P.ixocarpa* (variety Likhtaryk)*;* C1, C3 and E2 - *Petunia hybrida M1;* C2 and E3 - *Petunia hybrida 5P,* D1 and E1 - *Nicotiana tabacum* cv. Wisconsin,D2 *and* D3 - *Nicotiana benthamiana*.