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Proposed Protocol for Agrobacterium-mediated Transformation of Tomato Cotyledons for HSP101

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Protocol status: Other

This proposed protocol is based on established principles of *Agrobacterium*-mediated plant transformation; however, the exact workflow has not yet been experimentally performed or optimized by the author. Therefore, successful transformation and regeneration are not guaranteed and must be validated under approved laboratory conditions.

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Disclaimer

This protocol is a proposed educational workflow and has not been experimentally validated by the author. Any implementation must be conducted only in an approved laboratory under qualified supervision and in compliance with institutional biosafety and GMO regulations.

Abstract

This protocol describes the transformation of tomato (*Solanum lycopersicum*) cotyledons using *Agrobacterium tumefaciens* strain LBA4404 carrying the binary vector pPBV[Exp]-Bar-(CaMV 35S)>ath_HSP101. The method allows stable integration of the *ath_HSP101* gene under the CaMV 35S promoter



Guidelines

This protocol is intended as a proposed educational and research-planning workflow for future laboratory validation. It has not yet been experimentally validated by the author. All experimental implementation should be conducted only in an approved laboratory under supervision of qualified personnel and in accordance with institutional biosafety procedures.

All plant tissue culture work should be performed using aseptic technique to reduce contamination. Media, instruments, culture vessels, and work surfaces should be sterilized before use. Explants should be handled gently to avoid excessive tissue damage, as this may reduce regeneration efficiency.

The transformation efficiency and regeneration response may vary depending on tomato cultivar, seedling age, explant condition, bacterial culture condition, antibiotic concentration, and plant growth regulator composition. Therefore, optimization may be required before successful transformation and regeneration can be achieved.

Putative transformed plants should not be considered confirmed transgenic plants until molecular confirmation has been completed. Suggested confirmation methods may include PCR-based detection of the inserted gene and/or additional molecular analysis where available.

All genetically modified plant material, bacterial cultures, and contaminated consumables should be handled, contained, and disposed of according to institutional biosafety and GMO waste-management requirements.



Materials

Biological materials

- *Solanum lycopersicum* seedlings (7–10 days old)
- *Agrobacterium tumefaciens* LBA4404 containing pPBV[Exp]-Bar-(CaMV 35S)>ath_HSP101

Reagents

- LB broth
- LB agar
- Kanamycin
- Cefotaxime
- Murashige and Skoog (MS) medium
- Agar
- Sucrose
- Acetosyringone
- Silwet-L77
- Sterile distilled water

Plant growth regulators

- BAP (6-benzylaminopurine)
- NAA (α -naphthaleneacetic acid)
- IAA (indole-3-acetic acid)

Equipment

- Orbital shaker incubator
- Centrifuge
- Laminar flow hood
- Growth chamber or greenhouse
- Petri dishes
- Culture flasks
- Autoclave



Safety warnings

⚠ This proposed protocol involves *Agrobacterium tumefaciens*, antibiotics, and genetically modified plant material. It must only be performed in an approved laboratory under proper biosafety supervision and in compliance with GMO regulations.

Ethics statement

This protocol does not involve human participants or animals. Therefore, human research ethics approval and animal ethics approval are not applicable.

However, this protocol involves genetically modified plant material and *Agrobacterium tumefaciens*. Before experimental implementation, approval should be obtained from the relevant institutional biosafety committee, GMO committee, or equivalent regulatory authority. All work should be conducted in accordance with local regulations for genetically modified organisms, plant biotechnology research, biological containment, and environmental safety.

If institutional approval is obtained in the future, the name of the approving committee, approval number, permit number, and approval date should be added to this protocol before experimental work begins.

Before start

Prepare all culture media and sterilize materials before beginning the experiment. Work under sterile conditions inside a laminar flow hood when handling plant tissues.

Ensure the following materials are ready:

- Tomato seedlings (7–10 days old)
- *Agrobacterium tumefaciens* LBA4404 carrying the HSP101 vector
- LB media and antibiotics
- Murashige and Skoog (MS) plant culture media
- Plant growth regulators

Media preparation

1 LB broth (1 L)

1.1 Dissolve:

- 10 g tryptone
- 5 g yeast extract
- 10 g NaCl
- Adjust pH to **7.0**.
- Autoclave at **121 °C for 15–20 min**.

2 Kanamycin stock solution

- 2.1
- Dissolve **500 mg kanamycin in 10 mL sterile water**.
 - Filter sterilize using a **0.22 µm filter**.
 - Store at **–20 °C**.
 - Use **50 µg/mL** final concentration in LB media.

Procedure

3 Preparation of *Agrobacterium* culture

- 3.1
- Streak *Agrobacterium tumefaciens* LBA4404 on **LB agar + kanamycin (50 µg/mL)**.
 - Incubate plates at **28 °C for 48–72 h**.
 - Select a single colony and inoculate into **5–10 mL LB broth + kanamycin**.
 - Incubate at **28 °C, 200–250 rpm for 16–24 h**.
 - Transfer **1.5 mL of culture into 50 mL LB broth + kanamycin** and incubate again until turbid.

4 Preparation of transformation suspension

- 4.1
- Centrifuge bacterial culture at **4000–5000 rpm for 10 min**.
 - Discard the supernatant.
 - Resuspend the pellet in:
 1. **5% sucrose solution**
 2. **0.05% Silwet-L77**

5 Preparation of tomato cotyledon explants

- 5.1
- Germinate tomato seeds under sterile conditions.
 - After **7–10 days**, excise cotyledons from seedlings.
 - Cut cotyledons into small sections to expose wounded tissue suitable for infection.

6 Infection of explants

- 6.1
- Immerse cotyledon explants in the *Agrobacterium* suspension.
 - Incubate for **10–15 minutes** with gentle agitation.
 - Remove explants and blot dry on sterile filter paper.

7 Co-cultivation

- 7.1
- Transfer infected explants to **co-cultivation medium** containing:
 1. MS medium
 2. 100 µM acetosyringone
 - Incubate explants at **25 °C in darkness** for **2–3 days**.

8 Selection and shoot regeneration

- 8.1
- Transfer explants to **selection medium** containing:
 1. MS medium
 2. 50 mg/L kanamycin
 3. 250 mg/L cefotaxime
 4. 3% sucrose
 5. 0.8% agar
 - Add plant growth regulators:
 1. 1 mg/L BAP
 2. 0.1 mg/L NAA
 - Incubate under **16 h light / 8 h dark photoperiod at 25 °C**. (Shoots should emerge after **3–4 weeks**.)

9 Root induction

- 9.1
- Transfer regenerated shoots to **rooting medium** containing:
 1. ½ MS medium
 2. 0.5 mg/L IAA
 3. 3% sucrose
 - Roots typically develop within **1–2 weeks**.



10 Acclimatization

- ### 10.1
- Transfer rooted plants to soil pots.
 - Maintain plants in a greenhouse with controlled humidity.
 - Gradually acclimate plants to normal environmental conditions.