

Protocol for the *Agrobacterium*-mediated transformation of barley (*Hordeum vulgare*; cv. Golden Promise)

Introduction

This protocol is based on Tingay *et al.* (1997). It is designed to provide an uncomplicated and reproducible method for barley transformation, which can be readily transferred between labs. The protocol is intended to be reliable, not necessarily the most efficient. However, the overall simplicity greatly aids maximum throughput with minimal staff input.

We originally used bialaphos as our selectable agent, but have found hygromycin, as used in rice and, subsequently, barley transformation, to be very effective. Microscopic examination of the explants during tissue culture, although time consuming, greatly assists the initial selection of viable transformed callus lines. Monitor explants regularly to identify any excessive *Agrobacterium* overgrowth and discard affected embryos. The use of fresh media and antibiotic stocks is vital – prepare promptly and avoid storing antibiotic containing media for prolonged periods.

Please feel free to modify the protocol – we would be interested to hear of your improvements. Should you require any further information, please contact us via e-mail at enquiries@bract.org

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Diary of events

Day 1 Collect barley grains, approx 14 days post-anthesis. Sterilise by rinsing in 70% (v/v) ethanol, followed by 5 min in 50% (v/v) Sodium hypochlorite with 5 rinses of sterile water. Isolate immature embryos (1.5 – 2 mm diameter) from the grains, remove axis and transfer to callus induction medium (BCI; Wan and Lemaux, 1994; 25 embryos per plate), scutellum side up and incubate in the dark at 24°C.

Prepare an overnight *Agrobacterium* broth by adding a standard inoculum (Tingay *et al.*, 1997) of *Agrobacterium* to 10 ml of liquid MG/L medium (Garfinkel and Nester, 1980) - no antibiotics. Incubate for 20 h at 28°C, on a shaker (180 rpm).

Day 2 Using a pipette, drip full strength *Agrobacterium* suspension onto each embryo. Drag the embryo (gently!) across the surface of the medium to remove any excess *Agrobacterium* and transfer to fresh callus induction medium, scutellum side down. Incubate in the dark at 24°C and co-cultivate for 3 days. Discard any damaged embryos.

Day 5 Transfer embryos to callus induction medium + 150 mg l⁻¹ Timentin + selective agent and incubate in the dark at 24°C. Subculture the developing calli every 14 days, for a period of 12 - 24 weeks, following a suitable shoot regeneration programme (e.g. Harwood *et al.*, 2000). Only transfer resistant embryogenic lines to regeneration medium and discard any material stained with oxidised polyphenols. Immediately discard any explants which become overgrown with *Agrobacterium*.

This protocol should be read in conjunction with the enclosed poster, first published at the 10th International Association for Plant Tissue Culture & Biotechnology Congress, USA, 2002.

Barley regeneration media

Overview

Four types of media are required, which may be grouped into two pairs for ease of production.

Barley callus induction and rooting media are essentially of the same composition, differing only in the presence or absence, respectively, of growth regulator.

Shoot initiation and regeneration media are also similar (although different from the above) differing only in the presence or absence, respectively, of growth regulators and copper.

Appropriate selective agents and/ or antibiotics should be included for transformation experiments.

Barley callus induction (BCI)

4.3 g l⁻¹ Murashige & Skoog plant salt base

30 g l⁻¹ Maltose

1.0 g l⁻¹ Casein hydrolysate

350 mg l⁻¹ Myo-inositol

690 mg l⁻¹ Proline

1.0 mg l⁻¹ Thiamine HCl

2.5 mg l⁻¹ Dicamba

pH 5.8

3.5 g l⁻¹ Phytigel

Shoot initiation/ transition

2.7 g l⁻¹ Murashige & Skoog modified plant salt base (without NH₄NO₃)
20 g l⁻¹ Maltose
165 mg l⁻¹ NH₄NO₃
1.25 mg l⁻¹ CuSO₄·5H₂O
750 mg l⁻¹ Glutamine (reduce to 50 mg l⁻¹ if using Bialaphos as selective agent)
100 mg l⁻¹ Myo-inositol
0.4 mg l⁻¹ Thiamine HCl
2.5 mg l⁻¹ 2,4D
0.1 mg l⁻¹ BAP

pH 5.8
3.5 g l⁻¹ Phytigel

Shoot regeneration

2.7 g l⁻¹ Murashige & Skoog modified plant salt base (without NH₄NO₃)
20 g l⁻¹ Maltose
165 mg l⁻¹ NH₄NO₃
750 mg l⁻¹ Glutamine (reduce to 50 mg l⁻¹ if using Bialaphos as selective agent)
100 mg l⁻¹ Myo-inositol
0.4 mg l⁻¹ Thiamine HCl

pH 5.8
3.5 g l⁻¹ Phytigel

Rooting

4.3 g l⁻¹ Murashige & Skoog plant salt base

30 g l⁻¹ Maltose

1.0 g l⁻¹ Casein hydrolysate

350 mg l⁻¹ Myo-inositol

690 mg l⁻¹ Proline

1.0 mg l⁻¹ Thiamine HCl

pH 5.8

3.5 g l⁻¹ Phytigel

Agrobacterium medium

MG/L

5.0g l⁻¹ Tryptone

5.0g l⁻¹ Mannitol

2.5g l⁻¹ Yeast extract

1.0g l⁻¹ L-glutamic acid

250 mg l⁻¹ KH₂PO₄

100 mg l⁻¹ NaCl

100 mg l⁻¹ MgSO₄·7H₂O

10 µl Biotin (0.1mg/ml stock)

pH 7.0

15 g l⁻¹ agar (for plates)

References

- Garfinkel, M. and Nester, E.W. (1980) *Journal of Bacteriology* 144: 732-743
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- Wan, Y. and Lemaux, P.G. (1994) Generation of large numbers of independently transformed fertile barley plants. *Plant Physiology* 104: 37-38.