Barley transformation at SCRI

Jackie Lyon and Jennifer Stephens, Functional Genomics Facility
SCRI, Invergowrie, Dundee DD2 5DA.

Agrobacterium-mediated transformation of barley has become more efficient in recent years with the development of new methods and protocols, making high-throughput experiments more achievable. We routinely use the barley cultivar 'Golden Promise' to transform as it is currently the most amenable. We are testing the regeneration potential and transformation efficiency of other recalcitrant cultivars for future development.

Barley Transformation Protocol

Fig. 1. Tiller at 12 days post anthesis

Barley donor plants are grown in a controlled environment where temperature, light and humidity are closely monitored. Tillers are collected at the correct stage of development - approximately 12 days post anthesis (Fig. 1). The seed coat is removed to allow the immature embryo to be isolated and the axis taken off (Fig. 2). The embryos are co-cultivated with *Agrobacterium* cells containing the transformation vector for 3 days in the dark (Fig. 3). Embryos are then transferred to fresh media every 2 weeks to induce the development of callus (Fig. 4) and

Fig. 2. Barley embryo isolation

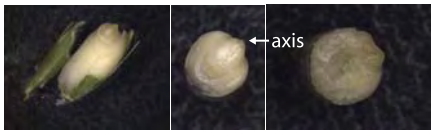


Fig. 3. Agrobacterium co-cultivation

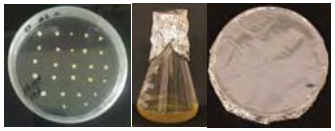


Fig. 4. Callus induction

Fig. 5. Regeneration of plants

subsequently moved on to regeneration media until they produce shoots (Fig. 5). Once the shoots have developed strong root systems they are transferred to soil in the glasshouse (Fig. 6). The whole procedure from collecting tillers to producing glasshouse grown transgenic plants takes around 4 months.

Fig. 6. Transgenic barley growing in the glasshouse

Screening of transgenic plants

All our transformants are confirmed by PCR for the selection gene (usually hygromycin) and the transgene (Fig. 7). In a test experiment using pBract204 vector (Fig. 8) we investigated the expression of GUS in various tissues (leaf, callus and seedlings; Fig. 9).

Fig. 7. PCR screening of transgenic plants

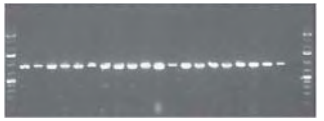


Fig. 8. pBract204 vector

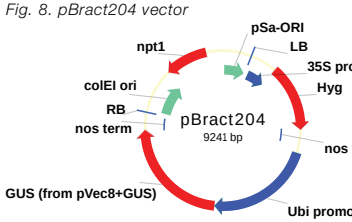
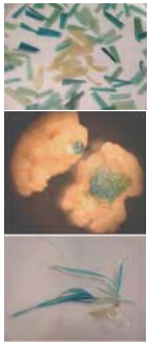


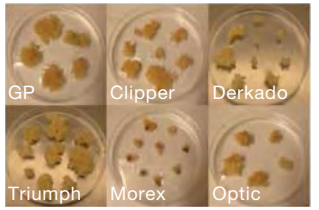
Fig. 9. GUS expression in transgenic barley



Regeneration of recalcitrant cultivars

We have tested a range of chemicals as supplements in our media and found improved callus induction (Fig. 10) and regeneration of some commercially important cultivars.

Fig. 10. Callus induction in other cultivars



Acknowledgments

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