

Long term storage of *Lotus corniculatus* root cultures and rescue of root cultures from transgenic plants

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One of the problems of working with transgenic legumes relates to the storage of a large number of transformed lines. At IGER we have produced a range of lines co-transformed with GUS (Webb *et al*, 1994), antisense DFR (Carron *et al*, 1994), antisense chalcone synthase (Colliver *et al*, 1994) and antisense ENOD2 (Skøt *et al*, 1996). Our preferred method for storing *L. corniculatus* lines transformed with *Agrobacterium rhizogenes* is to retain lines at 4°C.

Briefly, 1cm root tips from 'hairy roots' are grown on 0.8% agar supplemented with 1/2 B5 basal medium together with 3% sucrose. After 2-3 weeks at 25°C, plates are transferred to 4°C and then routinely subcultured every 6 months. However on occasions, stored root cultures can be lost due to infection, cold room breakdown etc. We present here a simple method for rescuing valuable root culture lines from derived glasshouse-grown plants.

Materials and methods

Take young stem or petiole segments, approximately 1cm in length, from glasshouse-grown plants. Decontaminate by soaking for 10 minutes in 20% sodium hypochlorite. Then wash several times in sterile distilled and deionised water and discard any stem sections with excessive bleaching at cut ends.

Place segments on 1/2 B5 plates supplemented with 3% sucrose but no antibiotics and place in dark at 25°C. After 2-4 weeks 'hairy roots' should be noted growing from the ends of the segments and these can then be excised and cultured as normal 'hairy roots'.

Comments

This method has allowed us to recover *Lotus* lines harbouring constructs in binary vectors. We do not know however whether this procedure promotes the silencing of transgene expression but this could be easily tested by comparing levels of antibiotic resistance, kanamycin or hygromycin, in stock root cultures and in root cultures rescued from derived transgenic plants.

References

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