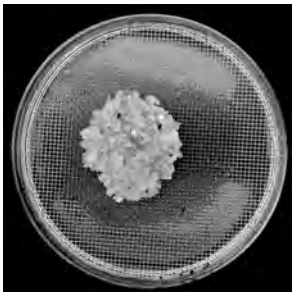
Nylon mesh as an improved support for bombarded calli or cell suspensions

S.J. Dalton, P. Robson, M. Buanafina, A.J.E. Bettany, E. Timms, D. Wiffen and P. Morris
Institute of Grassland & Environmental Research, Aberystwyth, Wales, UK. SY23 3EB
Email: sue.dalton@bbsrc.ac.uk

Keywords: genetic transformation, *Poa, Lolium, Festuca*



Introduction Using cell suspensions to transform some grass species by particle bombardment has a number of disadvantages including increased somoclonal variation in liquid cell culture and poor performance due to polysaccharide production. The use of calli avoids these problems, but the manipulation of calli through numerous media changes is laborious and time-consuming. We investigated a possible mechanism to facilitate the use of calli in transformation by immobilising calli on mesh.

Materials and methods A range of nylon meshes from 0.5-2mm (Cadisch) were tested to look at callus formation in pre-arranged targets. Embryogenic primary calli were transferred onto petri-dish sized mesh circles placed on callus medium and were arranged to form a target. After three to seven days growth the meshes were transferred to high osmolarity medium prior to bombardment in a particle inflow gun using parameters which varied depending on species and tissue. The following day the meshes were transferred back to callus induction medium and typically a week later the calli were transferred from the mesh to solid selection media. Cell suspension cultures were also tested and either plated to form pre-arranged targets on mesh as with calli and after bombardment were subsequently treated as callus, or were bombarded as usual, returned to cell suspension for initial selection and only plated onto mesh for regeneration. The results in Table 1 were collated over a large number of co-transformation experiments involving a number of genes of interest and two selection systems.

Results The calli of *Lolium multiflorum, Agrostis stolonifera, Festuca rubra* and *Poa pratensis* formed a thick mat of callus and grew through the mesh, leaving very little tissue behind when the mesh was moved. By contrast *Festuca arundinacea*, *Lolium perenne* and *Lolium temulentum* calli did not grow through the mesh and small pieces of callus were frequently left behind after transfer. Mesh of 1mm (Figure 1) proved best overall (data not shown). Enmeshed calli could be bombarded at higher pressures without disturbing the callus. The calli remained in the same orientation throughout recovery, whereas calli which are disturbed by bombardment may land with the bombarded surface face down in the medium and thus lose embryogenic potential. For those species where there is no alternative to callus, mesh was more convenient to use even if regeneration rates were not much improved (Table 1). In *F.arundinacea* and *L.multiflorum* which form good cell suspensions, better results were achieved with cell suspensions than with calli, whether plated on mesh to form a target or plated on mesh during selection. This allowed the suspension colonies to dry out slightly and encouraged embryogenesis.

Table 1 Regeneration of transgenic grass plants from bombarded callus and suspension cultures grown on mesh

Species and Tissue	Mesh	Successful bombardments	Percentage	No. of plants	No. plants per bombardment	Selection
F.arundinacea. Callus target	mesh	1/16	6	2	0.1	*CaMV-hpt*
Suspension as callus target	mesh	14/74	19	14	1.9	*CaMV-hpt*
Suspension plated during selection	control	45/56	80	102	1.8	*CaMV-hpt*
	mesh	16/16	100	38	2.4	*CaMV-hpt*
L.multiflorum Callus target	mesh	0/25	0	0	0	*CaMV-hpt*
Suspension as callus target	mesh	12/31	39	20	0.6	*CaMV-hpt*
Suspension plated during selection	control	21/63	33	34	0.5	*CaMV-hpt*
	mesh	6/18	33	8	0.4	*CaMV-hpt*
L.perenne callus target	control	3/17	18	3	0.2	*CaMV-hpt*
	mesh	7/45	15	8	0.2	*CaMV-hpt*
P.pratensis callus target	control	1/24	4	1	<0.1	*Ubi-nptII*
	mesh	13/78	17	30	0.4	*Ubi-nptII*

Conclusions The use of mesh to immobilise calli improves transformation by allowing large numbers of targets to be prepared, and by simplifying manipulations it allows treatments to be timed more precisely. Mesh also improves transformation in some cell suspensions. The technique was generally applicable but has proved particularly useful for the transformation of *Lolium.perenne, Poa pratensis, Festuca rubra* and *Agrostis stolonifera.*