Plant Cell Reports (1988) 7:459–462

Plant Cell Reports

A method for the rapid production of fine plant cell suspension cultures

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Received June 13, 1988/Revised version received September 29, 1988 – Communicated by E. W. Weiler

ABSTRACT

A method has been devised which allows the rapid production of fine suspension cultures of small aggregate size from suspension cultures of large average aggregate size, such as those of Capsicum frutescens. The method, which uses a Waring blender for aseptic homogenisation of cultures, has also been shown to be effective in rapidly producing suspension cultures from callus cultures. The suspension cultures so produced are particularly useful for immobilisation, such as in porous polyurethane foam matrices.

INTRODUCTION

The advantages of immobilisation of plant cell cultures are well documented, especially for the production of secondary metabolites (Brodelius et al. 1979; Lindsey and Yeoman 1984). In particular, immobilisation in reticulate polyurethane foam matrices has been shown to give enhanced production of the flavour compound capsaicin from Capsicum frutescens cultures, in addition to offering solutions to some of the problems of working with plant cell cultures on a larger scale (Mavituna et al. 1987a). This in situ method of immobilisation is dependent on the filtering of the plant cell aggregates into the stationary porous foam matrix, by the movement of the bulk liquid within the culture vessel. Once physically entrapped, the aggregates grow to fill the rest of the available space within the foams (Mavituna et al. 1987b). However, initial entry of the plant cells into the foams requires the plant cell culture to be in the form of a fine suspension. Suspension cultures such as those of Capsicum frutescens can be difficult to maintain in this form and tend to grow as cell aggregates of varying size, which may be greater than 3mm in some cases. A mechanical method has therefore been developed to reduce clumpy cultures of this kind to fine suspension cultures suitable for immobilisation. In addition to being far more rapid than selective culture transfer to give fine cultures, this method also avoids the possible disadvantages of chemical methods which may have other effects on cultures apart from the desired reduction in aggregate size.

The method has also been used to rapidly produce suspension cultures directly from callus cultures. Conventional techniques for the formation of a fine suspension culture from callus generally require several transfers in the liquid medium, over a period of many weeks. However, it is often preferable to maintain plant cell cultures in the form of callus, as the incidence of somaclonal variation is considerably lessened in the slower growing callus, as compared to suspension cultures. This is particularly important when using cell lines selected for a particular property, such as high production of secondary metabolites. The method described allows cultures to be kept in the form of callus and then to be reduced to a fine suspension culture as required for immobilisation. The speed and efficiency of the method as compared with conventional techniques, may also indicate its use as a general system for the production of fine cell suspensions from callus cultures.

MATERIALS AND METHODS

Plant Cell Cultures: Callus cultures of Capsicum frutescens Mill cv annum which had been selected for high yields of capsaicin were supplied by Professor M.M. Yeoman of the Department of Botany, Edinburgh University. The callus cultures were maintained on agar containing Schenk and Hildebrandt medium (Schenk and Hildebrandt 1972), at 25°C in continuous light. Suspension cultures were developed either by the inoculation of liquid medium with 1-2 g of callus and repeated transfer every 2-3 weeks or by the use of the homogenisation method described below. Suspension cultures were maintained in 60 ml of Schenk and Hildebrandt medium in 250 ml Erlenmeyer flasks in continuous light on an orbital shaker (100 rpm) at 25°C.

Homogenisation: To give a reduction in the size of the plant cell aggregates, 500 ml of normal suspension culture was homogenised in a sterile 1 litre Waring blender (Christison Scientific Ltd., Gateshead) at 13,000 rpm for 5 seconds. The production of suspension cultures from callus was effected by the aseptic transfer of approximately 30-40g of callus to a sterile 1 litre Waring blender vessel and homogenisation at 13,000 rpm for 10 seconds in 500 ml of Schenk and Hildebrandt medium. In both cases the homogenised culture was then poured into 250 ml Erlenmeyer flasks in 50 ml aliquots. Over the following period of 30 days one of the flasks was removed for sampling every few days and an analysis was made of fresh weight, dry weight, viability and aggregate size distribution.

Immobilisation: Cultures for immobilisation were placed in 250 ml Erlenmeyer flasks containing

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five 1 cm^3 polyurethane foam particles which were held stationary on a stainless steel wire. Flasks were periodically harvested for the analysis of fresh weight, dry weight and viability, of both the immobilised cells and any material remaining in suspension.

Viability: Viability was measured using the fluorescein diacetate viability test according to the method described by Widholm (1972).

Fresh and Dry Weights: Samples were filtered under vacuum and weighed immediately and after 24 hours drying at 100°C.

Aggregate Size Analysis: The range of aggregate sizes within samples was analysed by wet sieving, with the sample being sequentially washed through a series of meshes of decreasing pore size, from 3.00 - 0.25 mm (Park 1986).

Replication: All data shown are the average of three replications of the experiments. A high degree of reproducibility of all the results was found between replicates.

RESULTS AND DISCUSSION

The homogenisation of either callus or suspension cultures leads to the breakdown and complete loss of a considerable proportion of the plant tissue (up to 75%) and the homogenised culture is initially of low viability (30-45% for homogenised callus; 55-65% for homogenised suspension). However, the viability rapidly recovers over a period of approximately 10 days and approaches that of a suspension culture inoculated at time zero with a similar concentration of cells (Fig.1). The recovery period for the viability of the homogenised cultures corresponds to the lag period of the normal suspension culture. It might be expected that this recovery period of the homogenised cultures would also be followed by a lag period before the onset of accelerated growth, but this does not appear to be the case. Hence it can be seen in Fig.2 that the normal supsension culture and the homogenised cultures have initially very similar growth patterns. It is interesting to note that the biomass concentration measured in the homogenised callus culture is greater than in the other cultures. It also seems that the viability of homogenised callus culture is maintained at greater than 90% up to the 30th day.

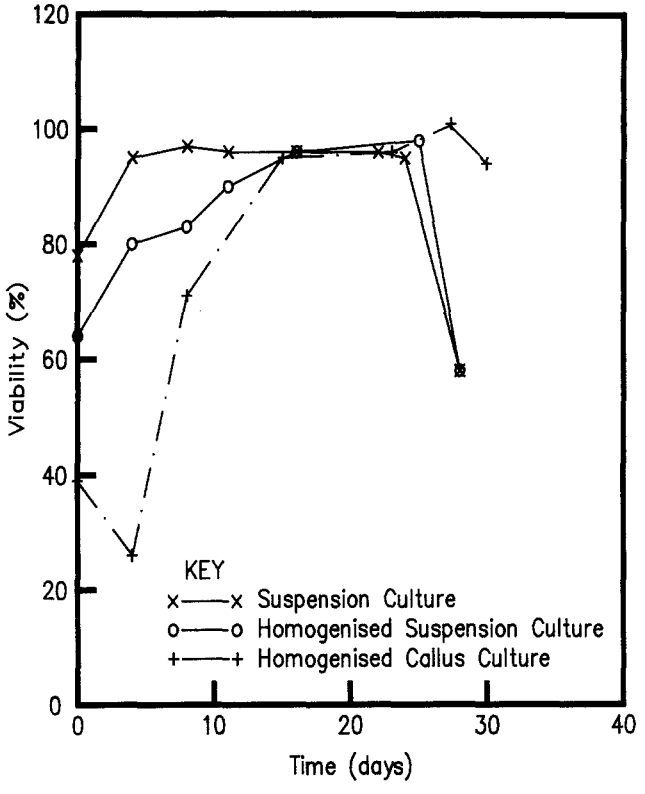


Figure 1 Percentage Viability of Normal Suspension, Homogenised Suspension Culture and Homogenised Callus Culture.

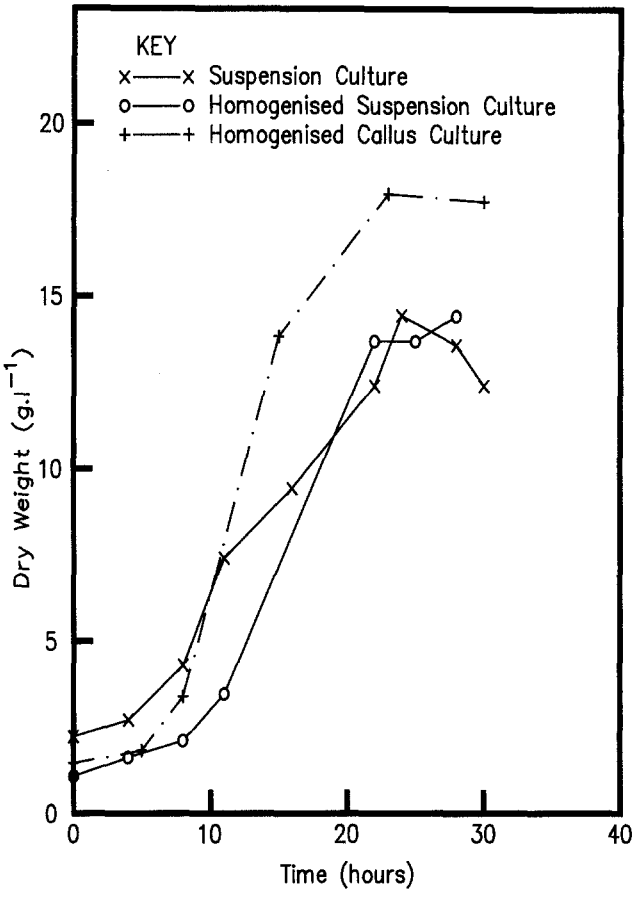
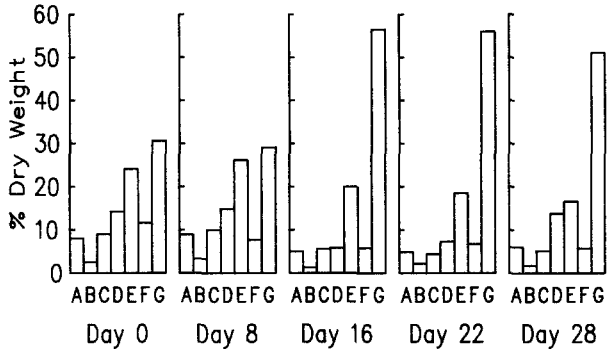
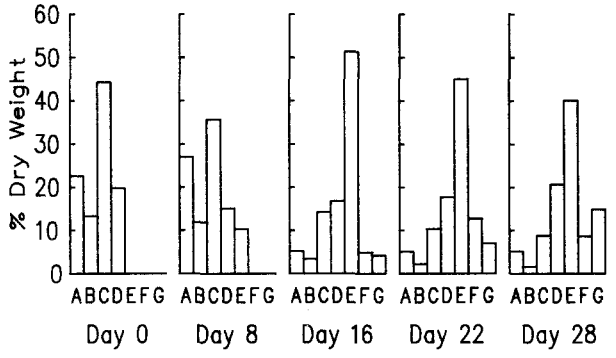


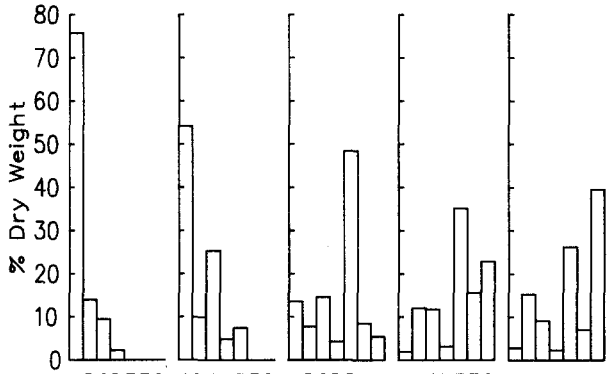
Figure 2 Growth Curves of Homogenised Cultures Compared with a Normal Suspension Culture.



Cell Aggregate Size Distribution of a Normal Suspension Culture



Cell Aggregate Size Distribution of a Homogenised Suspension Culture



Cell Aggregate Size Distribution of a Homogenised Callus Culture Growing as Suspension Culture

KEY : A<0.25mm, B 0.25–0.50mm, C 0.50–1.00mm, D 1.00–1.25mm, E 1.25–2.75mm, F 2.75–3.00mm, G>3.00mm

Figure 3 Changes in Cell Aggregate Size Distribution of Various Cultures During Growth Over 30 Days.

A variety of intracellular compounds including some toxic materials from the vacuoles would have been released into the medium during homogenisation. However, the presence of these toxic compounds does not seem to have a detrimental effect on growth.

Immediately after homogenisation the cultures are found to be made up of, on average, far smaller cell aggregates than are found in a normal suspension culture at a comparable stage (Fig.3). Figure 3 also shows that during the growth of both normal and homogenised cultures there is an increase in the average size of the aggregates. This is partly due to the non-separation of daughter cells during growth, but in the homogenised cultures may also be due to a process of reaggregation. However, it can be seen that, even after approximately 30 days of growth, both homogenised cultures remain substantially finer than the normal suspension culture. If it is required to maintain the homogenised cultures in their finest state, this can be done by selective subculturing during the period of active growth of the cultures.

Homogenised callus provides a fine culture which is ideal for entrapment methods of immobilisation, such as immobilisation in porous foams. Figure 4 shows that little biomass is retained in the foams, up to approximately 15 days, after which time the immobilisation of the homogenised callus within the foams rises rapidly compared to that of the normal

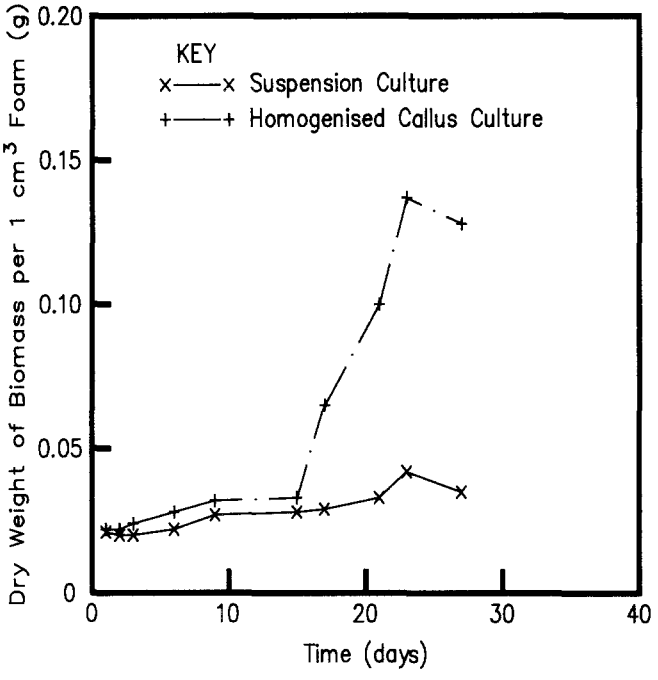


Figure 4 Immobilisation of Normal Suspension Culture and Homogenised Callus in Porous Foam Matrices.

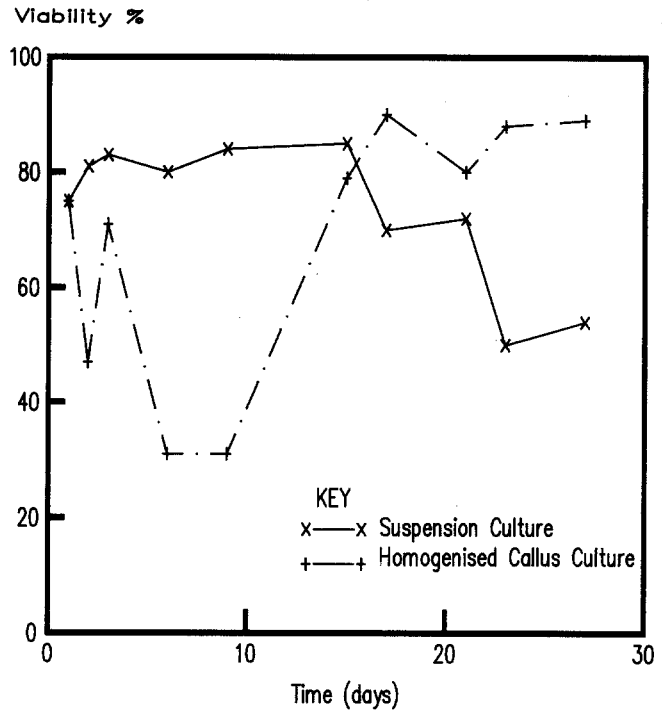


Figure 5 Viability of Immobilised Suspension Culture and Immobilised Homogenised Callus.

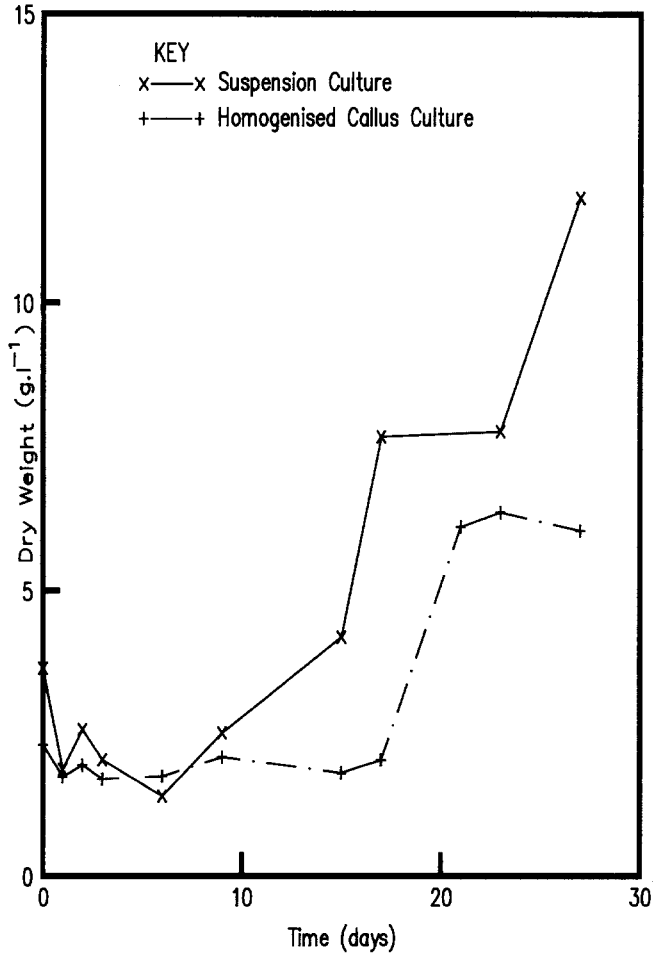


Figure 6 Biomass Remaining in Medium After Immobilisation of Normal Suspension and Homogenised Callus Culture.

suspension culture. The degree of immobilisation of the normal suspension culture is limited by the proportion of the culture consisting of small enough aggregates to penetrate the porous matrix. The homogenised culture can penetrate evenly throughout the whole of the foam, but is not retained until viability of the culture recovers (Fig.5) and cell growth and reaggregation begins. Once this stage is reached the homogenised culture is rapidly immobilised and the available space within the foams is filled. Consequently, the concentration of freely suspended biomass increases in the medium. The normal suspension culture has higher levels of biomass remaining in the liquid medium than does the homogenised culture, due to the less efficient immobilisation of the normal suspension culture (Fig.6).

CONCLUSION

Despite the initial loss of a proportion of the culture during homogenisation and the initially low viability of cultures so treated, the homogenisation technique seems to offer a number of advantages as a system for the production of fine suspensions of plant cells. In particular the application of the method to cultures required for immobilisation may be of benefit. The method has been successfully used in this laboratory with a wide variety of species (Capsicum frutescens, Catharanthus roseus, Mentha spicata, Mucuna pruriens, Nicotiana tabacum and Papaver somniferum) and does not appear to cause any long term damage to cultures.

ACKNOWLEDGEMENTS

The authors would like to thank the Biotechnology Directorate of the SERC and Albright and Wilson Ltd. for financial support.

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