Development of culture system suitable for micropropagation of rice via somatic embryogenesis

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1. Introduction

As one of the measures that can help solve the food crisis, the use of high-yield culture systems for the clonal mass propagation of important food plants has been receiving much attention. Successful *in vitro* somatic embryogenesis of various crops has been reported, including rice. In particular, efficient plant regeneration by somatic embryogenesis and the establishment of a system of high-frequency embryogenesis in suspension cultures have been investigated.

In terms of bioreactor design, the intensity and distribution of hydrodynamic stress generated by agitation or aeration are among the important factors that must be addressed. However, there have been few papers dealing with bioreactor design for rice callus culture. This is due to the fact that plant cells are generally weak against hydrodynamic stress. In this study, embryogenic rice calli were cultivated using three types of bioreactors and their regeneration frequency was investigated. One of them, called a turbine-blade reactor (TBR), has been developed for hairy root culture. In this reactor, the cultivation space is separated from the agitation space by a cylindrical stainless-steel mesh and a stainless-steel plate with a slit so that plant cells such as hairy roots or calli do not come into contact with the impeller. Immobilized culture of plant cell has been widely applied for the continuous production of secondary metabolites. Immobilization can protect plant cells from hydrodynamic and shear forces and can prevent the loss of plant cells in continuous mode. In order to overcome growth inhibition at a high agitation speed, we also investigated the use of polyurethane foam as an immobilization support material.

Next, the operational optimization of immobilized culture was carried out. In order to increase the immobilization efficiency, the effects of support volume, bioreactor operation and modification were analyzed. The regeneration frequency of immobilized rice callus was also checked. Finally, Effective regeneration culture system for embryogenic rice callus was investigated.

2. Results and Discussion

2-1. Development of a bioreactor suitable for embryogenic rice callus culture

Embryogenic rice calli induced from mature rice (Oryza sativa L., Sasanishiki) were cultured in a jar-fermentor with disk turbine impellers, an air-lift reactor, and a turbine-blade reactor (TBR), and the effects of agitation were investigated. In all cases, the growth inhibition was observed, though a slightly improved regeneration frequency was obtained in the TBR. To overcome the growth inhibition, small cubes of polyurethane foam were used as immobilization supports in the TBR culture. Supports accumulated around the cylindrical stainless-steel mesh in the reactor and rice calli were observed growing in them. Polyurethane foam with an average pore size of 1.3 mm gave the maximum ratio of immobilized cells during 1-week culture. When 5- or 10-mm cubes were used, supports were observed floating on the medium surface, but 3-mm cube supports accumulated uniformly around the stainless-steel mesh and were found to be suitable for the immobilized culture of rice calli. Three-millimeter cube supports corresponding to 5 % by volume were added to 600-ml medium in the TBR and the effects of the agitation speed on the cell growth and regeneration frequency were investigated. At all the agitation speeds examined, no significant decrease in either the cell growth or the regeneration frequency occurred. From these results, it was concluded that the TBR is a suitable reactor for the propagation of embryogenic rice calli and that immobilization supports with 1.3 mm of average pore size are effective in preventing hydrodynamic stress as well as in supplying nutrients to the immobilized calli.

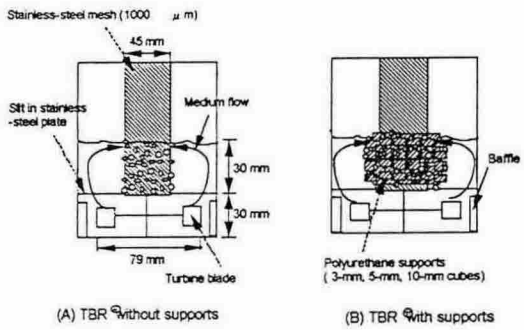

Fig. 1. Schematic diagrams of TBR without and with supports used in rice callus culture.

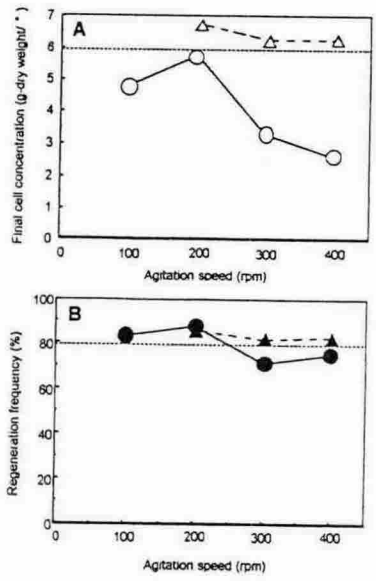

Fig. 2. Effect of agitation speed on cell concentration (A) and regeneration frequency (B) of rice calli in TBR without (○, ●) and with (△, ▲) supports. Dotted lines show the results of the control culture.

2-2. *Operational optimization of a TBR using macroporous support and its application to rice callus regeneration*

Optimal operation condition was investigated for immobilized rice callus culture using a turbine blade reactor (TBR) with polyurethane foam supports. By using polyurethane foam block as immobilization support, the inhibition of cell growth at a high agitation speed was avoided because the hydrodynamic stress against immobilized cell was probably reduced. Experimental results in each operational condition were assessed by means of rice callus growth, immobilization ratio in TBR and those regeneration frequencies in regeneration culture using solid medium. Concerning with pore size of polyurethane foam and support size, three-millimeter cube support of polyurethane foam with an average pore size of 1.3 mm was the most suitable support. The maximum immobilization ratio was 50 % under 5 % support volume by volume of growth medium. For improving the immobilization ratio of rice callus in the TBR, the optimum TBR operation and modification were investigated further. By repeating a periodic operation 3 times (agitating at 300 rpm for 5 min and then 50 rpm for 2 min, and then 200 rpm of constant agitation speed during the remaining time), almost all supports could entrap rice callus and homogeneous immobilization was attained. The immobilization ratio was improved as compared with that using a constant operation at 200 rpm. Next, the TBR was modified by setting an air sparger inside the stainless mesh cylinder. In the modified TBR, the floating support by air bubbles was reduced, and the immobilization ratio increased further and reached 86.3 % when we increased the support volume to 15 % under periodic operation on a daily basis. The regeneration frequency of immobilized callus was also slightly increased by periodic operation and modification of the TBR.

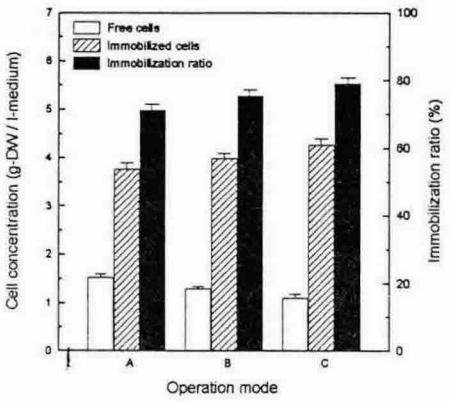

Fig. 3. Effect of bioreactor operation on immobilization ratio after 7day culture (support volume: 60 ml). A: constant agitation speed at 200 rpm; B: periodic operation (agitation speed 300 rpm for 5 min / 50 rpm for 2min) only on the first day (the periodic operation was repeated 3 times, and then 200 rpm during culture time); C: periodic operation on a daily basis (the periodic operation was repeated 3 times everyday). Data show the average of the two determinations; error bar shows the range of the two determinations.

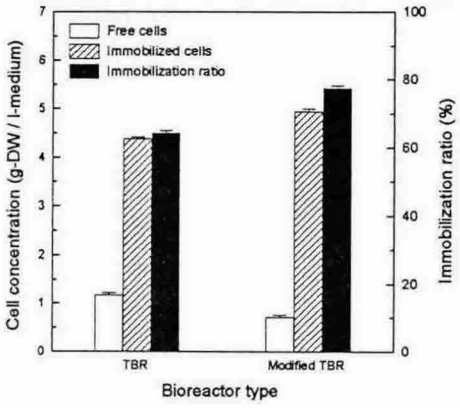

Fig. 4. Effect of bioreactor modification on growth and immobilization ratio after 7 day culture in the modified TBR under the periodic operation on a daily basis (support volume: 60 ml; inoculum: 6.7 g FW/l). Data show the average of the two determinations, error bar shows the range of the two determinations.

2-3. Development of two-stage regeneration culture system for embryogenic rice callus

Effective regeneration culture system for embryogenic rice callus was investigated. Solid regeneration culture has a small quantity of regenerated calli even though its regeneration frequency is relatively higher. And liquid regeneration culture tends to that regenerated calli make a lump during cultivation even though number of regenerated calli is relatively higher. In order to overcome these problems, we developed a two-stage regeneration culture system for embryogenic rice callus. In the two-stage regeneration culture, number of regenerated calli increased further and making a lump of regenerated calli was avoided. From these results, it is concluded that the two-stage regeneration culture system for embryogenic rice callus is effective.

3. Concluding Remarks

Based on the above views, we were studied the development of culture system suitable for micropropagation of rice via somatic embryogenesis in this study.

It was considered that these results can be helpful to develop the effective culture system for embryogenic rice calli. One the basis of these results, the following novel regeneration system is proposed. Immobilization supports are transferred directly into the regeneration medium and the regenerated calli are obtained in situ in the supports. Regenerated calli in the supports with 3 to 5 germinated shoots are incubated in a preculture nursery and then transferred to a normal paddy. This system is attractive since it is easily applicable to conventional agricultural procedures. In order to realize the system, further experiments relating to regeneration with immobilized supports and scale-up of the bioreactor need to be carried out.

4. References

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