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Plant regeneration from cultured protoplasts of a glutinous rice

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ABSTRACT

Young embryos of rice (*Oryza sativa* L. subsp, japonica var. Guo-xiang No.I) were cultured on MS agar medium(2,4-D 2 mg/l). Calli were formed and subcultured on N6 agar medium (2,4-D 2 mg/l). After selection, the small, grainy and pale yellowish cell clusters with dense cytoplasm were used in protoplast preparation. Isolated protoplasts were cultured in N6 medium(2,4-D 1 mg/l, 6-BA 0.2 mg/l)[1]* with agarose block culture method. The protoplasts grew, divided and formed calli. After inducing differentiation, the regenerated mature plants were obtained.

Key words: *protoplast culture, Oryza sativa, glutinous variety, plant regeneration.*

INTRODUCTION

Plant regeneration from rice protoplasts has been reported in recent years [1-14] These are important advances in protoplast culture. It is necessary to extend the experiences to other varieties and related species in *Oryza* in order to use them in somatic hybridization and genetic manipulation. After successful obtaining the regenerated plants from cultured protoplasts of rice variety-*Oryza sativa* subsp. japonica Nonghu No.6(Longhu No.6), the experiments of other variaties were also carried out in our laboratory [10-12] . In this paper, the results of protoplast culture of a glutinous rice variety will be described.

MATERIAL AND METHODS

The material used in this experiment was *Oryza sativa* subsp, japonica, var. Guo-

[1]* 2,4-D-2, 4-dichlorophenoxyacetic acid.
6- BA - 6- benzyladenine.

xiang No. 1 (Kao-shan No.l) which is a glutinous variety of rice cultured in East China. The plants were grown in phytotron (28℃, 12 hr. light/day, relative humidity 70%).

1. Establishment of calli

Young embryos (7-9, 15, or 20 days after anthesis) were cultured in MS agar medium(2,4-D 2 mg/l, lactoalbumin hydrolysate 300 mg/l. YE 300 mg/l or 2,4-D 2 mg/l, CM 5% :pH5.8)[1]*, 25℃, dark. After 20 days subculture, calli were formed and transferrd to N6 agar medium(2, 4-D 2 mg/l;pH 5.8) with 2 weeks interval. After 6 months subculture, it was ready for use in protoplast preparation.

2. Protoplast preparation

The small, grainy, pale yellowish cell clusters with dense cytoplasm were incubated in enzyme solution (EA_3-867 cellulase 1%, hemicellulase 2%, Macerozyme R-10 0.3%, dextran-K-sulphate 0.3%, MES 5.8 mg/l, NaH_2PO_4 10 mg/l, mannitol 0.5*M*; pH 5.8)z* in 28℃, 4hr. Then changed with fresh enzyme solution and again incubated in 28℃, 4-6 hr. Higher yield of protoplasts was obtained(Fig. 1).

3. Protoplast culture and plant regeneration

The agarose block culture method was used in protoplast culture.

Media:

Protoplast: N6 (2,4-D 1 mg/l, 6-BA 0.2 mg/l, glucose 0.45*M*, sucrose 1%, CM 5%, pH 5.8)

Callus subculture: MS (2,4-D2mg/l, YE 500 mg/l, CM 5%, agar 0.7%, pH 5.8).

Differentiation: MS (ZT 2 mg/1, KT 1 mg/l, agar 0.7%, pH 5.8)[3]*.

RESULTS AND DISCUSSION

1. In 3 days culture, the protoplasts were enlarged clearly, 50% of them became oval-shaped in 5 days culture. In 10 days culture, first cell divisions were observed, and average percentage of them was 0.04% in 15 days culture(Fig.2). Second cell divisions were observed in 20 days culture(Fig.3). The clusters which consisted of 8-12 cells were appeared in 30 days culture(Fig.4). At this time, fresh medium with lower glucose concentration(0.25 *M*) was added. After visible calli formed, the agarose blocks were transplanted on MS agar medium (the composition is similar to N6 medium but without glucose, and 0.06 *M* sucrose was added). After two months culture, again, the calli grew faster. While the calli were about 0.5 cm in diameter, they were transferred on differentiation medium (Fig.5). After selection, two kinds of calli could be distinguished. First, fast-growing, pale in color with loose texture, some aged gradually and some continued their growth in subculture.

[1]* YE-Yeast extract.
CM- Cocoanut milk.

[2]* MES-2(N-morpholino) ethane sulphonic acid

[3]* ZT- Zeatin.
KT- Kinetin.

Second, slow-grawing, pale-yellowish and grainy, somatic embryo like textures appeared in these calli(Fig.6). After observing with super thin sections and TEM, these cells Showed rich in cell contents with clear nucleus and other organelles (Fig. 7, 10). After transferring the calli of second type on differentiation medium, regenerated plants were obtained(Fig. 8, 9). The fertility of these plants was low. Various characteristics of second generation plants were still maintained, and details of paths in plants regeneration are being investigated.

2. The development state of the young embryo influenced calli initiation. The explants of different days after anthesis were compared. Best results were obtained in using embryos of 7-9 days after anthesis. The callus induction percentage is 100%. They grew well and faster, and suitable for the preparation of protoplasts.

3. In agarose block culture, too much liquid medium is undesirable. If liquid covered the agarose block, it is unfavorable to protoplast growth.

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Plate I

Fig. 1 Protoplasts isolated from calli (40 × 6.3 × 4)

Fig. 2 First cell division in 10 days culture. (40 × 6.3 ×l0)

Fig. 3 Second-third cell divisions in 20 days culture. (40 ×6.3 × 4)

Fig. 4 Clusters with 8-10 cells in 30 clays culture. (40 × 6.3 × 4)

Fig. 5 Callus about 0.5 cm in diameter. (15 × 6.3 × 4)

Fig. 6 Somatic embryos formed in the callus. (25 × 4)

Fig. 7 Super thin section of somatic embryo. (15 × 6.3 × 4)

Fig. 8 Regenerated plantlet from protoplast.

Fig. 9 Regenerated plants from protoplasts.

Fig. 10 Cell with the potency of differentiation. (X20,000)-