ISOLATION, FUSION AND MULTIPLICATION OF SUGARCANE PROTOPLASTS AND COMPARISON OF SEXUAL AND PARASEXUAL HYBRIDIZATION

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KEY WORDS

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SUMMARY

The problems associated with the isolation of leaf protoplasts in sugarcane have been overcome by the choice of spindle tissue as source material. It is now possible to isolate, fuse, regenerate and multiply sugarcane protoplasts. However, the usefulness is limited due to the shortlived chromosomal stability during the callus stage. Nevertheless, preliminary results are promising and the method should prove feasible with refinement in technique. Two breeding systems for using parasexual hybridization are discussed.

INTRODUCTION

The isolation of sugarcane protoplasts was first reported by MARETZKI & NICKELL (1973). Protoplasts from other plants have been isolated and regenerated by KAO et al. (1970) and GAMBORG et al. (1973). Fusion was first reported by COCKING (1972) and regeneration of para-sexual hybrids by CARLSON (1972). Since then a number of reports have appeared on fusion and regeneration (MELCHERS et al., 1974; GLEBA et al., 1974; KAO et al., 1973.

The present work describes briefly the procedures adopted in the isolation, fusion and regeneration of the sugarcane protoplasts and the potential of the system to support and enhance conventional breeding work. Parasexual hybridization and genetic transformations have to be considered in the light of shortlived chromosomal stability in the callus stage. Therefore, it would be desirable to assess the period of stability of the chromosomes and the approximate time cycles of mitosis for controlling the variations necessary for the development of economic plants.

MATERIALS AND METHODS

Sugarcane (Saccharum officinarum and S. spontaneum) sets or root stocks are grown in three litre planters. The plants are supplied with abundance of nutrients and water for lush growth. When the plants are growing vigorously, the desired plants are removed to a dark place for at least twelve hours prior to harvesting of the tops. The tops are harvested with the spindle and brought into the laboratory where they are dissected; the spindle is removed, taking about half a centimeter below the dome
(meristematic region) to three centimeters above. The thickness to be taken is judged by the softness of the spindle tissue. The sections are placed in a 200 ml/litre commercial hypochlorite solution and transferred to a bioclean station. All further operations are carried out under aseptic conditions.

The spindle tissues are washed free of hypochlorite in three changes of sterile distilled water after which both ends are cut and one layer of sheath tissue removed. The dissected tissue is sliced and left in medium ‘A’ containing 40 g/litre cellulase Onozuka R10; 10 g/litre macerozyme Onozuka R10; 10 g/litre pectinase Sigma; 6 mmol/litre calcium chloride; 1 mmol/litre calcium tetrahydrogen orthophosphate, 0.35 mol/litre sorbitol and 0.35 mol/litre mannitol (GAMBORG et al., 1973). The medium ‘A’ is sterilized by filtering through 40 μm millipore membrane. The sliced tissue is left in the medium ‘A’ for 180 minutes which is sufficient to digest the cell walls. The tissue is macerated with a glass rod, filtered through cloth and the medium ‘A’ removed by low speed centrifugation and washing twice with medium ‘B’ consisting of 0.4 mol/litre glucose, 3.5 mmol/litre calcium chloride, 0.7 mmol/litre potassium dihydrogen phosphate and 3 mmol/litre 2-(n-morpholino) ethane sulphonic acid (KAO et al., 1973). The protoplasts thus obtained are placed in round bottomed embryo blocks and medium ‘C’, each kg consisting of 500 g polyethylene glycol 6000 and 500 g medium ‘B’ is added slowly (KAO & MICHAYLUK, 1974). Adhesion is quick. The protoplasts are left in the medium ‘C’ for 30 minutes and then washed with modified Murashige-Skoog medium (KRISHNAMURTHI & TLASKAL, 1974). They are then cultured either on slides kept in Petri dishes with high humidity, or in 50 ml. Erlenmeyer flasks with a shallow layer of modified Murashige-Skoog liquid medium for good aeration. Desiccation or concentration of salts in the medium through evaporation should be prevented.

RESULTS

The enzyme mixture digests the cell walls and releases protoplasts between 150-180 minutes after the commencement of the treatment with medium ‘A’. The released protoplasts, after washing with medium ‘B’ and subjecting to medium ‘C’ adhere immediately. When the protoplasts are identical it is difficult to identify autofusion and fusion between protoplasts from separate sources. The protoplast used in our experiments are fortunately different. The S. officinarum ‘Badila’ protoplasts are darker in colour, the S. spontaneum is light and clear whereas Sclerostachya narenga is green and hence easy to identify the fusion between species (Fig. 3).

Fusion takes place in two stages. In the first place adhesion is immediate after adding polyethylene glycol 6000 mixture (Fig. 3). A number of protoplast clumps can be seen (Fig. 2). Fusion starts towards the end of 25 minutes period or as soon as polyethylene glycol mixture is washed (Fig. 4).

Fig. 1. Sugarcane protoplasts isolated from the spindle region.
Fig. 2. Adhesion of cells after adding polyethylene glycol.
Fig. 3. Adhesion between Saccharum officinarum (dark) and S. spontaneum (light) protoplast.
Fig. 4. The process of fusion.
Fig. 5. Two independent nuclei as a result of incomplete fusion.
Fig. 6. Cell division and wall formation.