Short Communication

Growth of Rice Root-derived Callus Tissue in Suspension Culture

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Callus tissue from rice root has been cultured in vitro by Yatazawa et al. (4, 5), Wu and Li (2), Yanagawa et al. (3), and Iyer and Raina (1). In each of these cases, the callus tissues were maintained on solid substrata and grown much too slowly for harvest at frequent intervals and in insufficient yield for many biochemical and physiological studies.

The present communication describes some of the characteristics of rice root-derived callus tissue growing continuously in suspension culture for the past 20 months in this laboratory. The cultures show a doubling time of 22 hr and plateau at 3 to 4 g wet weight, 10 to 11% of which is dry weight.

MATERIALS AND METHODS

Root Callus Induction. Callus formation was induced with 2,4-dichlorophenoxyacetic acid essentially as described by Yatazawa et al. (4). Rice seeds (Oryza sativa var. starbonnet) were surface sterilized by soaking the seeds in 50% commercial Clorox with 1% v/v Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) for 20 min. The seeds were washed with several changes of sterile deionized water and then distributed in groups of 2 among sterile Petri dishes containing filter paper and 10 ml of sterile deionized water. The seeds were allowed to germinate at 30 C in the dark. After 5 to 7 days, those seedlings showing no evidence of microbial contamination were transferred to a 0.6% agar medium containing 2,4-D at 2 mg/l in 250-ml flasks. These flasks were incubated in the dark at 30 C.

Callus Culture. Root callus tissues were excised with a sterile scalpel and incubated at 30 C in the dark on agar nutrient medium (4). The medium was contained in French-type square bottles adapted with teflon cap liners.

Suspension Cultures. Adaptation of the callus tissue to suspension culture was accomplished in a 100-ml jacketed culture vessel (spinner flask, Bellem Glass, Vineland, N.J., Cat No. 3006) aerated through the sidearm with cotton-filter sterilized air. The inoculum was obtained by washing the tissue from the surface of the agar medium (in French squares) with suspension medium. Suspension medium was prepared by omitting agar from the medium described by Yatazawa et al. (4). When it was estimated that the callus tissue had completed about four doublings in the spinner flask, the tissue was transferred to fresh medium in 250-ml Erlenmeyer flasks (100 ml/flask) and incubated in water bath shakers (New Brunswick Scientific Model G-76) maintained at 30 C and shaking at a rate of 180 to 200 rpm. Cultures were subcultured first weekly, and later twice weekly with inocula of approximately 500 mg wet weight.

RESULTS AND DISCUSSION

Induction and development of sufficiently large root callus tissue for subculture required 6 weeks of incubation on 2,4-D agar. The excised callus tissue was then cultured for 10 weeks on the Yatazawa medium with agar, and adaptation to the suspension medium required 8 weeks of incubation with constant, slow stirring and aeration. Once adapted to suspension growth, the tissue established a characteristic color, morphology, and rapid growth rate that has remained constant for more than 2 years. The tissue grows as small clumps of cells of 1 mm average diameter (about the size of the original root callus nodule). The tissue is buff colored during exponential growth and early stationary phase with slight darkening in late stationary phase.

The clumps sediment from the medium as soon as agitation is stopped. Subcultures from late stationary phase show a lag of 2 to 3 days before the characteristic rapid growth is re-established, whereas cultures transferred at 3- to 5-day intervals show no measurable lag.

Analyses of growth kinetic data show a doubling time of about 22 hr, even when analyses separated by 20 months are compared (Fig. 1). As noted by Yatazawa et al. (4), the growth rate is greatly reduced by prolonged autoclaving of the medium and filter-sterilized media support much more rapid growth. From the results of preliminary work on medium composition and filter sterilization, we should expect to reduce the doubling time to between 14 and 19 hr. Maximum yield from a 500-ml inoculum in 50 ml of autoclaved medium is 3 to 4 g wet weight of tissue in mid-stationary phase, and no additional growth could be induced by the addition of salts or vitamins; however, addition of sucrose at early stationary phase (96 hr) increased the maximum yield to 4 to 6 g. Analysis of the 96-hr medium by TLC showed no sucrose, glucose, or fructose, and such media clarified by centrifugation showed no carbohydrate in the anthrone assay, indicating that the plateau on the growth curve is due to carbon depletion.

Growth Measurements. Early stationary phase cultures, containing about 30 mg tissue/ml, were combined to give a tissue concentration of about 20 mg/ml, based on expected wet weight, in a sterile flask with a Teflon-coated magnetic stirring bar. Aliquots of 10 ml were taken with a large bore pipette while the tissue was maintained in suspension by the stirring bar and used as inocula for fresh medium. The cultures were incubated as described above and sacrificed at the times indicated. Tissue was harvested and washed with deionized water by vacuum filtration on a Buchner funnel. Wet weights were determined from tissue scraped from the filter paper, and dry weights were determined after the tissue had been dried 24 hr at 80 C.

1 Journal paper, New Jersey Agricultural Experiment Station. This work was supported by Regional Research Fund NE-53, United States Department of Agriculture.
GROWTH OF RICE CALLUS TISSUE

Sectioned and stained clumps show some cellular organization within the clumps. There is a broad band of cells near the center which appear more densely stained than those of the outermost or innermost regions, perhaps indicating that the clump has a specific meristematic region. All cells of the clump appear to be parenchymatous, and vascular tissue has not been detected in either thin sections or in crushed unstained preparations.

No secondary wall thickening was evident in any of the sections examined, and cells could be ruptured very easily. Homogenates have been prepared in a Ten Broeck type homogenizer that appear to represent greater than 90% breakage.

We realize that stability of the tissue, as indicated by doubling time and morphology, does not necessarily attest to genetic stability. However, we will report in a subsequent communication on our comparative studies of an enzyme in rice plants and rice callus cultures. Preliminary results indicate that enzyme levels and biochemical characteristics are similar if not identical to those of the parent tissue.

Acknowledgment—The authors wish to thank K. Simsay for technical assistance.

LITERATURE CITED