A simple rapid photometric estimation of the growth response of immobilized cells to fusaric acid and gamma radiation

Tal Tepper, Meira Ziv, and Amram Ashri

The Hebrew University of Jerusalem, The Otto Warburg Center for Biotechnology in Agriculture, P.O. Box 12, Rehovot 76100, Israel

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Abstract

A simple and rapid photometric method was developed in order to evaluate the growth responses of cells to various factors in vitro. Cells of Asparagus officinalis L. were mixed with autoclaved agar at 40°C and 90 µl drops were dispensed into several Petri dishes. After the drops solidified, they were covered with a liquid growth medium and incubated on a gyratory shaker. Growth was measured every 2 or 3 days by two methods. In the first, the agar drop was placed under a stereomicroscope with substage illumination and the light passing through the embedded cells was measured by a light-meter. Growth, expressed by the increase in cell density, was inversely proportional to the intensity of transmitted light. In the second method, the agar drop was melted in a microwave oven and the packed cell volume was measured. The correlation between the two methods showed that the photometric method can be used to assess growth response of immobilized cells, during the first two weeks of culture. This method was used to evaluate growth responses to the toxin fusaric acid and to gamma radiation. The photometric method requires a small amount of inoculum, standard microscopic equipment and can be used to determine the effect of various factors on the growth of intact plant cells in vitro without disruption.

Abbreviations. FA, fusaric acid; NAA, naphthyl-1-acetic acid; CH, casein hydrolysate; 2-l-P, N6-[2-isopentyl] adenine; 2,4-D, 2,4-dichloro-phenoxy acetic acid; PCV, packed cell volume; gy, grey.

Introduction

The utilization of cell cultures for the selection of desirable genotypes can be a valuable tool for the breeder, but it is still handicapped by technical problems (Wenzel 1985). Selection for resistance using inhibitors or toxins, requires precise definition of the exposure parameters of the cell culture. The factor used for the selection must be tested over a wide range of concentrations in order to determine the growth response of the cell populations. Cell sensitivity can be determined by several methods such as cell counting, fresh and dry weight, packed cell volume, mitotic index and viability staining. Some of these methods are laborious and cumbersome since they require large quantities of cells, several repetitions and tedious microscopic examinations. They also result in the disruption of the in situ cultured cells for constant sampling (Street 1973). The measurements are often very variable and may require sophisticated and expensive equipment. In many cases the sampling and measuring are not frequent enough and may result in erroneous conclusions (Gonzales and Widholm 1985).

This paper presents a simple, non-invasive, cell growth measuring method developed to determine the in vitro sensitivity of asparagus cells to fusaric acid and gamma radiation.

Materials and methods

Cell culture: A suspension culture of Asparagus officinalis L. was established from hypocotyl explants isolated from seedlings three to four weeks old, on MS (Murashige and Skoog 1962) agar medium containing 0.005% adenine sulphate, 3% sucrose and 5.0 µM 2,4-D. The callus was transferred to liquid cultures kept on a gyratory shaker (100 rpm) in 1000 ml Erlenmayer flasks containing 400 ml MS medium (MS-111), supplemented with 3% sucrose, 4% mannitol, 0.05% CH, 0.5 NAA, 1.0 2,4-D, 0.5 2-l-P (in µM) and adjusted to pH 5.6-5.8. Callus and cell suspension experiments were kept at 25±1°C under 30 µE·m⁻²·s⁻¹ while agar immobilized cultures were
kept under 0.15 μE·m⁻²·s⁻¹ of continuous light.

**Preparation of immobilized cells:** A cell fraction of 280–500 μm was collected using polyamide sieves, (Nybolt PA-500/57 and PA-280/51, Swiss Silk Bolting Cloth, Zurich) and was immobilized according to Steinbrenner et al. (1989). The collected cell fraction was suspended (at 1.5:1 ratio) in water. For immobilization, the cell suspension was mixed (at 1:2 ratio) with autoclaved MS-111 medium supplemented with 1.8% agar, cooled to 40°C to a final concentration of 0.6%. The cell agar mixture was stirred continuously and kept in a 40°C water bath. Five single 90 μl drops of the cell-agar mixture were dispensed into 5 cm Petri dishes and kept in a laminar hood at room temperature to allow solidification (Fig. 1).

**Fig 1: Immobilized cells in agar drops covered with liquid medium in a Petri dish, after one day.**

The arbitrary units of the light-meter were used as the measuring units. Before taking measurements in each Petri dish, the light was passed through the liquid medium and the light intensity was adjusted to a constant level. In order to assure that reduction in light transmittance was not due to changes in liquid or agar media, transmittance through a cell-free agar drop was measured throughout the culture period.

**Packed cell volume (PCV):** Cell growth was assessed every 2 or 3 days by measuring 5–10 agar drops. Each single agar drop was put in an Eppendorf tube containing 1 ml of distilled water. The tube was heated in a microwave oven for 1 min, then placed in a warm water bath (60–80°C) and the cells were allowed to sink for a 2 min period. The volume of the pellet was measured by replacing it with water.

**Gamma irradiation:** Agar drops were prepared as described and were covered by 4 ml liquid MS-111 medium. The next day the medium was removed and the Petri dishes were exposed to gamma radiation produced by 60Co source which delivered 4.17 gy·min⁻¹. Exposure time was adjusted to deliver doses of 0, 30, 80 and 140 gy, 2 Petri dishes per treatment. After 30 min, the agar drops were washed and covered by new MS-111 medium. The cultures were incubated as described above.

**Fusaric acid (FA) treatment:** Agar drops were prepared as described and covered with 4 ml liquid MS-111 medium containing FA (5-butyl-picolinic acid, Sigma) in concentrations of 0, 7, 11, 15 and 50 ppm, two to three dishes for each treatment.

**Results and discussion**

**Comparison between growth measurement by the photometric and packed cell volume methods:** Cell growth was assessed every 2 to 3 days by photometric measurements under a stereomicroscope. The agar drop containing the embedded cells was placed under the stereomicroscope so as to cover the whole field of view and the intensity of light that was transmitted through the agar drop was measured.

**Fig 3: Growth curves assessed by PCV and by the photometric methods.**