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SHORT COMMUNICATION

EFFECTS OF SUGARS ON INVERTASE ACTIVITY OF CARROT CELLS

C. P. P. RICARDO*, T. AP REES and W. A. FULLER
Botany School, University of Cambridge, Downing Street

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Key Word Index—*Daucus carota*; Umbelliferae; invertase; tissue cultures; effects of sugars.

Abstract—The development of acid invertase activity in both tissue cultures and disks of storage tissue of *Daucus carota* was not reduced by exogenous hexose.

WE HAVE proposed that acid and alkaline invertases contribute to the regulation of sucrose metabolism in carrots[1] (*Daucus carota* L.). The present work was done to see if the activities of these invertases varied according to whether carrot cells received sucrose or hexose.

Inocula from tissue cultures, established from explants of storage tissue, were grown on the following media: Hildebrandt's 'tobacco' medium[2] with either sucrose or a mixture of equal amounts of glucose and fructose as the principal source of carbon; Heller's medium[3] with the above sources of carbon; and a defined medium[4] with sucrose or glucose or fructose as the carbon source. The resulting calli were sub-cultured, each onto its own respective medium, and the cells that grew from these sub-cultures were assayed for invertase after growth for periods ranging from 5 to 13 weeks. Activity of alkaline invertase was not found in any culture. The cell-wall and the supernatant fractions of homogenates of all cultures contained appreciable acid invertase activity that was optimum at pH 4·5. Activity in the cell-wall fraction varied in parallel with that in the supernatant fraction. Total activities of acid invertase were in the range 300–600 mg reducing sugar formed per 2 hr/10 g fr. wt. For five different isolates, we found that total activities of acid invertase were similar in cells grown on sucrose and in corresponding cells grown on hexose.

In a second series of experiments cultures were grown on paper rafts on 20 ml Heller's medium (liquid) that contained sucrose or a mixture of equal amounts of glucose and fructose as the source of carbon. After 6 weeks the media were replaced so that cells previously grown on sucrose were given hexose, and cells previously grown on hexose were given sucrose. Four days later the cultures were assayed for total acid invertase activity. Transfer from sucrose to hexose or vice versa had no demonstrable effect upon acid invertase activity. Finally, we report that incubation, under aseptic conditions, of disks (1 × 10 mm) of storage tissue of carrot in 0·05 M glucose, as opposed to water, for 72 hr did not consistently affect the development of acid invertase activity that occurs under these conditions.[1] We conclude that acid invertase of carrot cells can develop in the presence of considerable

* Present address: Departamento de Fisiologia Vegetal, Estação Agronómica Nacional, Oeiras, Portugal

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quantities of exogenous hexose and that provision of sucrose instead of hexose does not necessarily enhance this development.

EXPERIMENTAL

Unless stated otherwise tissue cultures were grown at 25° on solid media until fr. wt had increased 10–20-fold. Media in which hexose was the main source of carbon were made by substituting equivalent amounts of glucose, fructose, or glucose and fructose in equal amounts, for the sucrose components. Sucrose was removed from any coconut milk, that was used to prepare hexose media, by treatment with purified invertase. Disks of storage tissue were prepared as described previously[5] and were aged at 25° by shaking in 200 ml solution in 2-1 Erlenmeyer flasks plugged with cotton wool. Homogenates, cell-wall and supernatant fractions were prepared and assayed for invertase as described previously[1] except that the extraction buffer was 0·187 M Na_2HPO_4—0·006 M citric acid buffer (pH 7·6) that contained Polyclar AT (insoluble polyvinylpyrolidione) at 250 mg per g tissue.

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