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BRIEF COMMUNICATION

Sucrose Synthesis in Callus Cultures

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Abstract. Occurrence and operation of sucrose synthetic system in randomly selected callus cultures such as persimmon, soybean and poplar cultures were examined by ^{14}C-tracer analysis and determining enzyme activities involved in sucrose metabolism. All the enzymes examined were present and ^{14}C-glucose was transformed into ^{14}C-sucrose in every callus. Sucrose synthetic capacity appears to be widely distributed in cultured plant tissues.

Sucrose is the usual carbohydrate present in plant tissue culture media. Thus its utilization in explant, callus, cell and organ cultures has been examined by several researchers (MARETZKI *et al.* 1974, THORPE 1982). In contrast, sucrose biosynthesis in culture has been studied very rarely, probably due to the greater concern with its utilization as a carbon and energy source *in vitro*. The earliest study showed that carrot callus readily interconverted glucose and fructose, and synthesized the disaccharide when supplied with either of the monosaccharides (GORIS 1954). The question whether or not the cultured tissues in general synthesize sucrose and the mechanism of the biosynthesis have remained relatively unexplored. We have examined some aspects of sucrose metabolism in Japanese morning-glory callus, including the uptake, biosynthesis and degradation (HISAJIMA 1975, 1978). It was found, *inter alia*, that ^{14}C-sucrose was synthesized in morning-glory callus after ^{14}C-glucose administration. In the present study, persimmon, poplar and soybean callus cultures were randomly chosen as experimental materials and sucrose metabolism was examined by determining the presence or absence of enzymes involved in sucrose metabolism. We have also determined the sucrose synthetic capacity of these tissues by feeding ^{14}C-glucose.

Persimmon (*Diospyros kaki* THUMB.) and soybean (*Glycine max* MERRILL.) cells were cultured on sucrose-containing medium according to the method

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TABLE 1
Cellular distribution and optimum pH of enzyme involved in sucrose metabolism

Enzymes	Callus tissue		
	Persimmon	Soybean	Poplar
Cytosol			
UDPglucose pyrophosphorylase	535 (8.0)	615 (7.8)	445 (8.0)
Sucrose synthase	180 (8.5)	221 (8.5)	212 (8.5)
Sucrose phosphate synthase	46 (6.5)	56 (6.0)	71 (6.5)
Invertase (acid)	19 (4.5)	18 (4.0)	58 (4.5)
Invertase (neutral)	176 (7.0)	156 (7.5)	181 (7.5)
Cell wall			
Invertase (acid)	178 (4.5)	355 (4.0)	225 (4.5)

* Enzyme activity is shown in [milliunit g^{-1} (f.m.)]. 1 milliunit was defined as the amount of enzyme which hydrolyzed 1 nmol of sucrose or formed 1 nmol of sucrose, sucrose phosphate or UDPG in 1 min under the standard assay conditions.
** Optimum pH is shown in parentheses.

described previously (HISAJIMA 1978). Poplar (*Populus alba* L.) cells were cultured according to the same method as soybean cells. Three week-old cells were used for experiments. ^{14}C-glucose administration and enzymatic experiments were made according to the method described previously (HISAJIMA and ARAI 1978, HISAJIMA 1979).

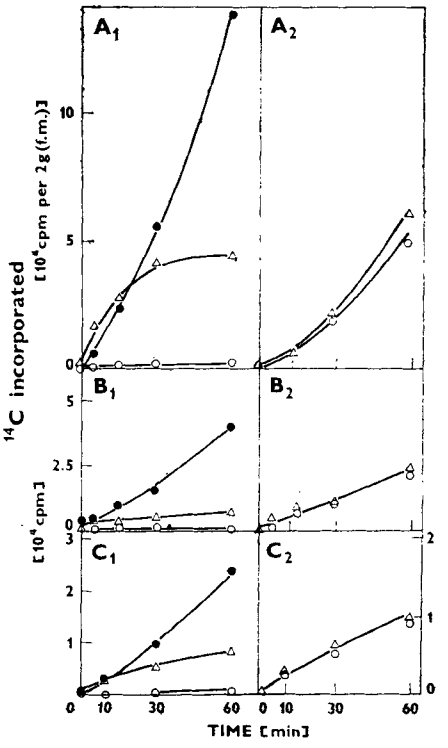

Fig. 1. ^{14}C-Glucose administration to callus cells. — A: Persimmon; B: soybean; C: poplar. (1), ^{14}C in free sugars; (2), ^{14}C in glucose and fructose moieties of sucrose. — Open circles: fructose; full circles: sucrose; triangles: glucose.

The presence and cellular distribution of some enzymes involved in sucrose metabolism are summarized in Table 1. In cell wall fraction, only acid invertase was detected. In the cytosolic fraction of callus of the three tissues examined soluble invertase (E.C. 3.2.1 26), UDPGppase (E.C. 2.7.7.9), sucrose synthase (E.C. 2.3.1.13), and sucrose phosphate synthase (E.C. 2.3.1.14) were present. Some of these cytosolic enzymes have been observed in other culture tissues, but only from Japanese morning-glory have all of them been reported (HISAJIMA 1975, 1979). Accordingly, enzymes capable of sucrose synthesis were present in the cytosol.

The results of ^{14}C-tracer experiments on the occurrence and operation of the sucrose synthetic system are given in Fig. 1. After ^{14}C-glucose administration to 3 week-old cells of each callus, the label appeared mainly in sucrose, in which it increased rapidly. In contrast, the label in glucose appeared to plateau early. It is clear, therefore, that the sucrose synthetic system works in callus of persimmon, soybean and poplar, which had been grown on sucrose-containing medium prior to incubation in ^{14}C-glucose. In morning-glory cells, ^{14}C-glucose was converted to sucrose through the culture period, suggesting that sucrose synthetic system works throughout the period (HISAJIMA 1979). Taking into account that the present results were obtained from randomly selected plant species, a similar sucrose synthetic system appears to be present and to work in wide varieties of plant callus cultures.

Fructose was labelled very weakly throughout the ^{14}C administration period in the present tissues. The glucose and fructose moieties of sucrose were nearly equally labelled before some label began to appear in free fructose. This suggests that free fructose is not involved in sucrose biosynthesis. It would thus appear that sucrose was not synthesized *via* the sucrose synthase system but rather *via* the sucrose phosphate synthase system. To date the presence and activity of sucrose phosphate synthase has not been thoroughly examined in cultured tissues. It has been characterized from Japanese morning-glory callus (HISAJIMA *et al.* 1980).

Results similar to the above have been obtained previously with Japanese morning-glory cells (HISAJIMA 1975, 1979). In this tissue, it was suggested that (1) medium sucrose was hydrolyzed by cell wall-bound invertase and then incorporated as monosaccharides, (2) that glucose was taken up preferentially over fructose by the cells, and (3) that sucrose was resynthesized *via* the sucrose phosphate synthase system. A preliminary experiment revealed that culture medium of the present callus cultures contained fructose, glucose and sucrose when the tissues were grown on sucrose-containing medium. A similar series of the above reactions may account for the data obtained. In some cases the bulk of the medium sucrose is reported to be taken up into the cells without prior hydrolysis (MARETZKI *et al.* 1974, THORPE 1982).

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BOOK REVIEW

LANGE, O. L., NOBEL, P. S., OSMOND, C. B., ZIEGLER, H. (ed.): PHYSIOLOGICAL PLANT ECOLOGY III. RESPONSES TO THE CHEMICAL AND BIOLOGICAL ENVIRONMENT. — Springer - Verlag, Berlin—Heidelberg—New York 1983. 799 pp. DM 298,—; approx. US $ 128.50.

The twelfth volume of the New Series of Encyclopedia of Plant Physiology consists of four parts, the first of which deals with features of the physical environment (Biol. Plant. 26 (2) : 155, 1984), and the next one with the special responses of land plants to water use and carbon dioxide assimilation. The volume reviewed considers specific aspects of the chemical and biological environment with special emphasis on the soil. The first part of the book dealing with physiological and ecological aspects of plant nutrition begins with the chapter on the significance of proton movements, ion uptake and other transport processes for fundamental properties of plant cells. In the following chapter physical principles related to osmoregulation and osmotic pressure generation are discussed. The next two chapters deal with osmotic adjustment and related salt uptake as an important component of salinity response of halotolerant eukaryotes, and with the direct biochemical effects of electrolytes in non vascular halophytes and halotolerant prokaryotes. The importance of nitrogen nutrition for plant growth is treated in chapter five, and the influence of limestone, silicates and soil pH on plant distribution in chapter six. In the next one referring on metal toxicity and tolerance of plants to metals significance of ligand properties rather than atomic mass *per se* is emphasized. The next group of three chapters is devoted to ecophysiology of nitrogen-fixing systems, mycorrhizal symbioses and lichens symbioses, which involve a complex of complementary organic and inorganic metabolism just as the interaction between plants and animals in marine systems (in chapter 11). The transfer of nutrients from consumed insects to carnivorous plants, which is mediated by secretion rich in proteins and energy, is dealt with in chapter 12. Chapter 13, which is the shortest of the book, treats the delicate balance in host-parasite systems. In chapter 14 the plant-virus relations are discussed, stress being laid on the "struggle" of genes. Chapters 15—17 are devoted to ecophysiology of zoophilic pollination, physiological ecology of fruits and their seeds and to herbivory as a powerful ecological interaction, its importance, plant responses to it, plant-herbivore symbiosis and evolution. The concluding chapter concerning the above-ground as well as underground plant-to-plant interactions is the logical transition of this part to the last one, dealing with ecosystem processes, mineral cycling, productivity and man's influence. Each chapter is complemented by full text references, and the volume is concluded by the author, taxonomic and subject indexes.

The book is well edited, keeping the well-known standard of presentation of this series. This volume in itself as well as the whole series is a must for every library of plant physiology department of the universities and research institutes.

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