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Growth Requirements of *Cuscuta reflexa*

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Abstract. Studies under *in vitro* conditions with *Cuscuta* stem tips have revealed that the parasite depends on the host for the supply of all essential requirements for its growth. The vestigial photosynthetic apparatus in *Cuscuta* cannot meet its total requirements. Thus for supply of energy it depends primarily on the host. Inorganic nutrients seem to be of secondary importance and perhaps help to some extent in its independent metabolic pool. Carbohydrates, particularly sugars, are presumably tapped by the parasite from the translocating channels of the host and further utilised for its own growth. Besides sugars, which form the carbon and energy source, amino acids and growth substances are also found to be utilised. The mechanism of carbohydrate utilization in *Cuscuta* has been postulated.

Cuscuta reflexa Roxb., being an angiospermic parasite, depends almost completely on the host plant for nutrients and water. It has a multiple host range of varied metabolism. The condition of the host will, therefore, affect the growth of *Cuscuta* under natural conditions and the host-parasite reaction could also decide some preferential hosts for it and its own mode of development and growth. For the proper understanding of the basic growth requirements of *Cuscuta* and its interlink with the host organ, culture technique (Loo 1945, Maheshwari and Baldev 1960, 1961, Baldev 1962 and Zimmermann 1962) has been applied. In the present study the main aim was to understand the mechanism of carbohydrate utilization and other organic nutrients in *Cuscuta*.

Materials and Methods

In the present study stem tips of actively growing *Cuscuta* filaments growing on *Duranta plumeiri* were selected. The stem tips, about 20 cm in length, were cut with a sharp blade under water and were immediately brought to the laboratory. These tips were then cut into pieces of 4 cm each under water and were surface sterilized with 80% ethyl alcohol for one minute. They were then transferred aseptically to culture tubes containing

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10 ml sterilized inorganic nutrient medium (ARNON and HOAGLAND 1943). The chemical composition of the inorganic nutrient medium referred to hereafter as "basic medium" (B.M.) is given below: $Ca(NO_3)_2 . 4H_2O$ — 236 mg, $MgSO_4 . 7H_2O$ — 36 mg, KNO_3 — 81 mg, KCl — 65 mg, KH_2PO_4 — 20 mg, ferric tartarate — 1.5 mg.

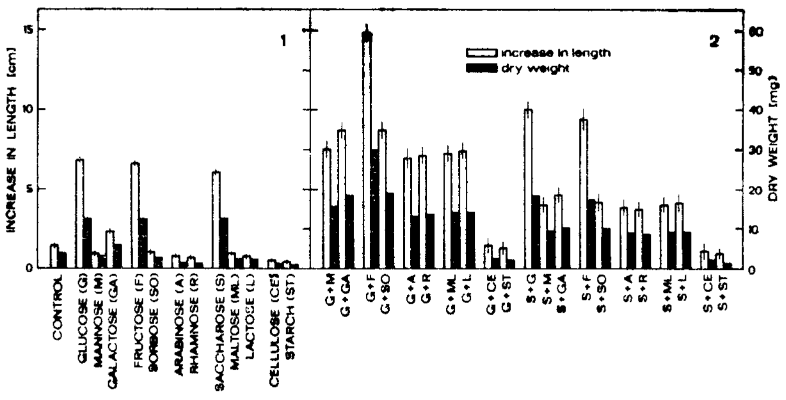
The basic medium was supplemented with organic compounds which are accepted as intermediate metabolites in the metabolism of higher plants: Carbohydrates, sugar alcohols, organic acids and amino acids in 1 % concentration, and growth substances in 0.0001 % concentration (REINERT 1963). Analar grade chemicals and bidistilled water were used throughout these studies.

Increase in the length of the stem tip and its corresponding dry weight during a period of four days were taken as the criteria of growth under various treatments. Several replicates were taken and the results were statistically analysed (SNEDECOR 1956).

Results

Effect of Various Carbohydrates and Sugar Alcohols on the Growth of *Cuscuta* Stem Tips *in vitro*

Results indicate (Fig. 1) that the growth of *Cuscuta* stem tips was most responsive to the monosaccharides, glucose and fructose. When these were combined in equal proportion, the effect was further enhanced (Fig. 2). Sucrose also responded almost equally well (Fig. 1), but sucrose in combination with glucose or fructose did not produce any significant effect as when a mixture



Figs. 1, 2. Effects of various sugars applied individually (Fig. 1) or in combination of two (Fig. 2).

of monosaccharides was used. The combined effect of glucose, fructose and sucrose (Fig. 3) was, however, not significantly higher than the combined effect of glucose and fructose only (Fig. 2).

As far as other carbohydrates are concerned, only galactose brought a significant increase in growth over the control while other carbohydrates were either ineffective or detrimental. The combined effects of glucose, fructose and sucrose with other carbohydrates brought increased growth due to the basic supply of glucose, fructose and sucrose. Thus, from the point of view of carbon source, *Cuscuta* prefers the latter three sugars over any other form of carbohydrates.

Fig. 4 depicts the effects of sugar alcohols either singly or in combination with glucose, fructose and sucrose whose favourable influence hase already been established (Fig. 1). Results indicate that the sugar alcohols could not

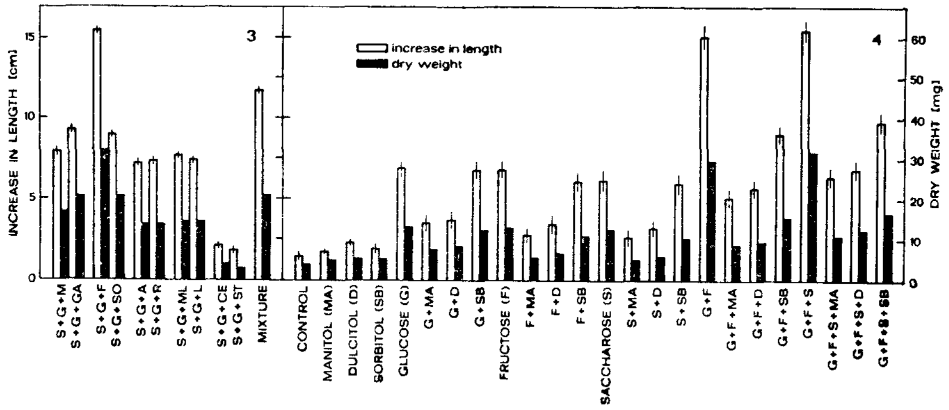


Fig. 3. Effect of various sugars applied in combination of three.

Fig. 4. Effect of sugar alcohols and sugar plus sugar alcohols on the growth of *Cuscuta* stem *in vitro*.

improve the growth of *Cuscuta* stem tips, either singly or in combination with the aforesaid sugars. Whatever growth was observed seemed to be due to the basic supply of glucose, sucrose and fructose. Contrariwise, the sugar alcohols brought inhibition in many cases.

Effect of Organic Acids on the Growth of *Cuscuta* Stem Tips *in vitro*

Results obtained by supplementing the inorganic salts with various organic acids (acetic, citric, oxalic, malic, malonic, succinic and tartaric acids) at a concentration of 1% of the basic inorganic medium, were not favourable (Fig. 5). On the other hand, if the basic inorganic medium was first supplemented with glucose and then with the organic acid, the organic acids did bring a favourable change in the linear growth over and above the single glucose effect. The linear growth effect is not confirmed by increase in dry weight. This causes some doubt regarding a permanent change in *Cuscuta* stem due to organic acids, other than an increase in size. Nevertheless, it is obvious that the primary requirement for *Cuscuta* growth is a sugar which to some extent may be further augmented by some of the organic acids of the Krebs cycle.

Effect of Various Amino Acids on the Growth of *Cuscuta* Stem Tips *in vitro*

Amino acids have been used by several authors (Loo 1945, Ramkrishnan 1963) to stimulate growth in tissue culture. Amino acids are essentially needed for the growth of both plant and animal organs. The present tissue culture study using eleven amino acids (glycine, alanine, valine, isoleucine, cystine, aspartic acid, asparagin, glutamic acid, histine and phenylalanine), at the uniform concentration of 1% of the inorganic basic medium have proved to be beneficial, in general. The only exception were alanine, histidine and phenylalanine. The increased growth was recorded both for the linear growth

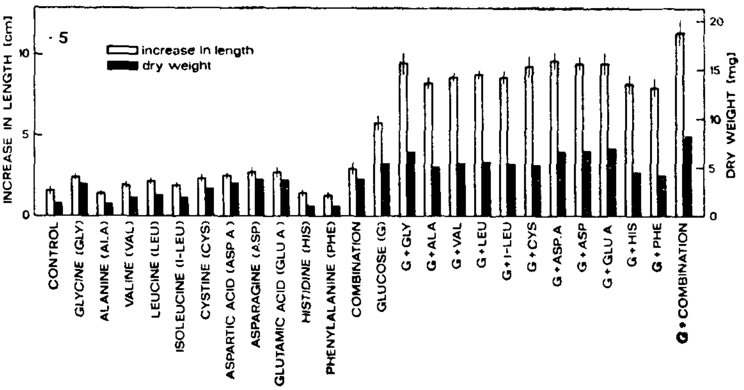


Fig. 5. Effect of various amino acids and amino acids plus glucose on the growth of *Cuscuta* stem *in vitro*.

as well as for the dry weight. When sugar was supplemented along with the amino acids, the linear growth was more effective than the dry weight increase. Here again, the effects of histidine and phenylalanine were not significant. The most effective ones were glycine, aspartic acid, asparagine and glutamic acid. A combination of all the aforesaid amino acids was, however, most stimulatory and particularly with the sugar.

It can be inferred (Figs. 1 to 6) that the primary energy source for *Cuscuta* is perhaps one of the sugars which could be a monosaccharide like glucose or fructose or a disaccharide like sucrose. Once the primary requirement is met, growth could further be stimulated by the organic acids of Krebs cycle, other fatty acids, and the common amino acids of the plant metabolism.

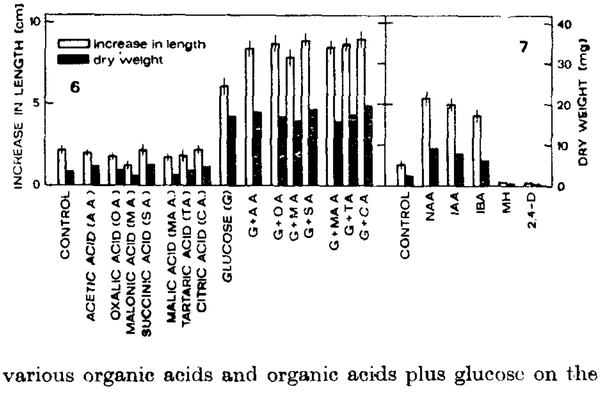


Fig. 6. Effect of various organic acids and organic acids plus glucose on the growth of *Cuscuta* stem *in vitro*.

Fig. 7. Effect of various growth substances on the growth of *Cuscuta* stem *in vitro*. — α-NAA: napthaleneacetic acid, IAA: indole-3-acetic acid, IBA: indole-3-butyric acid, MH: maleic hydrazide, 2,4-D: 2,4-dichlorophenoxyacetic acid.

Effect of Some Plant Growth Substances on the Growth of *Cuscuta* Stem Tips *in vitro*

Increased growth of the stem by the supply of plant growth substances has been demonstrated by numerous workers (LEOPOLD 1955). The favourable effects of growth substances have been reported by LOO (1945) in case of *Asparagus* tissues. In the present investigation α-NAA IAA and IBA at 1 ppm concentration (Fig. 7) have been found to enhance the growth of *Cuscuta* stem. Nevertheless, α-NAA was most effective (303% over the control). The increase in length was accompanied by an increase in dry weight. On the other hand, maleic hydrazide (MH) and 2,4-dichlorophenoxyacetic acid (2,4-D) were found to be not at all favourable for the *Cuscuta* growth.

Discussion

1. Nutritional requirements of *Cuscuta reflexa* under *in vitro* conditions:

Inorganic nutrients alone could not bring any appreciable change in the growth of *Cuscuta* stem tips, but when these were supplemented with sugars like glucose, fructose and sucrose they were found to be most effective in inducing better growth under *in vitro* conditions. From this it appears that sugars are readily preferred as a carbon and energy source by *Cuscuta*. Amino acids can partially replace sugar but work better in combination with the sugars than separately. Following the Embden-Mayerhof Parnas' pathway for the breakdown of glucose, fructose or sucrose, the intermediary role of amino acids is understandable. Both sugars and amino acids can be considered as energy sources which work more efficiently in combination and are eventually available to the parasite.

Sugar alcohols, on the other hand, are ineffective in increasing growth. HILDERBRANDT and ROKER (1949) have also reported similar findings. The deleterious effects on the absorption of water of these soluble nonelectrolytes *in vitro* can be due to their inhibitory action on the entry of water (COLLIANDER and BARLUND 1933). Under no condition have organic acids without sugar been found to accelerate the growth of *Cuscuta*. Organic acids are only the intermediate metabolites of sugar breakdown. These experiments could not establish their value as a nutrient for *Cuscuta*.

2. Growth relations:

A definite increase in length and dry matter production of the *Cuscuta* stem show that the growth promoting chemicals (IAA, NAA and IBA) have increased the uptake of minerals and synthetic reactions in the plant, bringing a change both in length as well as in dry weight. MH and 2,4-D were found to be detrimental to *Cuscuta* stem growth. From the foregoing findings it appears that *Cuscuta* growth is governed by some sort of hormonal mechanism.

3. Growth requirements of *Cuscuta reflexa* as revealed from tissue culture studies:

Studies under *in vitro* conditions with *Cuscuta* stem tips have revealed that the parasite depends on the host for the supply of all essential requirements for its growth. The vestigial photosynthetic apparatus in *Cuscuta* cannot meet its total requirements. Thus for supply of energy it depends primarily on the host. Inorganic nutrients seem to be of secondary importance and perhaps help to some extent in its independent metabolic pool.

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BOOK REVIEW

PATTEN, B. C. (ed.): **Systems Analysis and Simulation in Ecology.** Vol. III. — Acad. Press, New York—San Francisco—London 1975. 601 pp., $ 39,50.

The International Biological Programme (IBP), terminated in 1974, demonstrated that many fundamental problems concerning organisms and their environment require a comprehensive and interdepartmental research for their solution. The progress in most biological disciplines, including ecology, leads to decreasing the diversity of approaches and methods as well as to reducing the dispersion of problems arisen from different scientific schools. Hence, the necessity to integrate and coordinate investigations between the countries of the world and to focus attention on the central themes. One of the main themes, promoted by IBP, was the investigation of productivity of the major biomes of the world, which might facilitate the identification of potential uses of the existing and new natural resources. Modeling, simulation and system analysis very early proved to be integral parts of these investigations and became significant components of many national programmes of IBP. The main natural biomes, the range of problems they present and approaches to their solution were important items also of the U.S. IBP Analysis Ecosystems Programme. Some results of these systems analyses are reported in the volume under review.

The book contains 12 studies (divided into two parts) that grew out of the contributions presented at the symposium "Modeling and analysis of ecosystems" held at the University of Georgia in 1973. In Part I, the system models of grassland (by G. S. Innis), eastern deciduous forest (by R. V. O'Neill), desert (by D. W. Goodall), tundra (by P. C. Miller et al.) and coniferous forest (by W. S. Overton) are described. The last study of this part (by K. E. F. Watt) is devoted to the critique and comparison of biome ecosystem modeling. Part II deals mainly with fresh-water ecosystems grading into the estuarine model under the stress of human impact.

Individual models introduced differ with respect to their conception and operation involving interactions, processes and mechanisms of various orders and time and spatial scales. They do not portray the final resolution but characterize the state-of-the art in ecosystem modeling through its development. They give basic information on possibilities and capabilities of modeling and simulation technique as applied to the macrosystems and evidence that large bio-ecological systems would pose new problems of complexity and unmanageability necessitating new fundamental progress in system science.

The book is of a good editorial standard: it is well arranged, supplied with many figures, tables, schemes and computer records and completed with a detailed subject index. It successfully increases the number of books published on the initiative of IBP and is highly reccommended to specialists in various scientific fields.

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