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STUDIES ON THE MORPHOGENESIS OF ASPARAGUS

VI. Effect of sugar on callus and organ formation in the *in vitro* culture of shoot segments of the seedlings

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Introduction

To obtain a fundamental knowledge of the morphogenesis of asparagus, various kinds of tissues have been cultured *in vitro* in relation to organ and callus formation. The authors have carried out tissue culture of asparagus and have mainly studied the effect of auxins and cytokinins on the callus and organ formation.[4,5,6,7,12,13,14,15] In the present paper, some experimental results will be reported on the effect of sugars on the callus and organ formation.

In the majority of cases in the tissue culture of asparagus as in that of various kinds of other plants, 1 to 4% of sugars were added to the culture media.[1,2,3,8,16] In this experiment, the effect of sucrose, glucose and fructose added to culture media on the callus and organ formation were investigated through the *in vitro* culture of segments excised from the asparagus seedlings.

Materials and Methods

The seeds of asparagus (*Asparagus officinalis* L., cv. Mary Washington 500) were germinated on agar media under aseptic conditions. When the first shoot of seedlings became 5 cm in length, segments 1 cm long were excised from the internode just below the first node, and were cultured on solid media. Number of segments cultured were 3 per 100-ml flask, and 30 per batch.

The media used contained MURASHIGE and SKOOG's inorganic and organic substances, 1 mg/l of NAA combined with 0.1 mg/l of BA, 7 g/l of agar and one of the sugars (sucrose, glucose and fructose) in which the concentrations were 0, 1, 2, 4, 8 and 16%. pH of the media were adjusted

to 5.5. After heating and pipeting 25 ml into 100-ml Erlenmeyer flasks, all media were autoclaved at 120°C for 15 min. The cultures were maintained at 25°C under 16-hour daily illumination (circa 4,000 lx with white fluorecent lamps).

Results and Discussion

In this experiment, the callus formation from the segments, and the redifferentiation of shoots and roots from the callus were observed. The callus was induced after 1 week of culture. Fig. 1 shows the percentage of callus-forming segments after 16 weeks of culture. It was 90 to 100% at 1 to 4% of the sugars. However, at 8% of the sugars, in the case of sucrose, the values were high, while they were low with glucose and fructose. Callus formation was hardly observed at 16% of the sugars.

The callus grew swiftly to become large at 2 or 4% of the sugars. The growth of the callus was not so good at 1, 8, and 16% of each sugars (Fig. 2). A slight difference was seen among callus formation for different sugars. It may be that, as far as sucrose, glucose and fructose are concerned, the concentrations of the sugars was more significant rather than the kind of sugars in relation to callus formation.

Shoots began to redifferentiate from the callus after 8 weeks of culture. In this experiment, all shoots redifferentiated from the callus. The percentage of shoot-redifferentiating callus clumps was highest at 2% of each

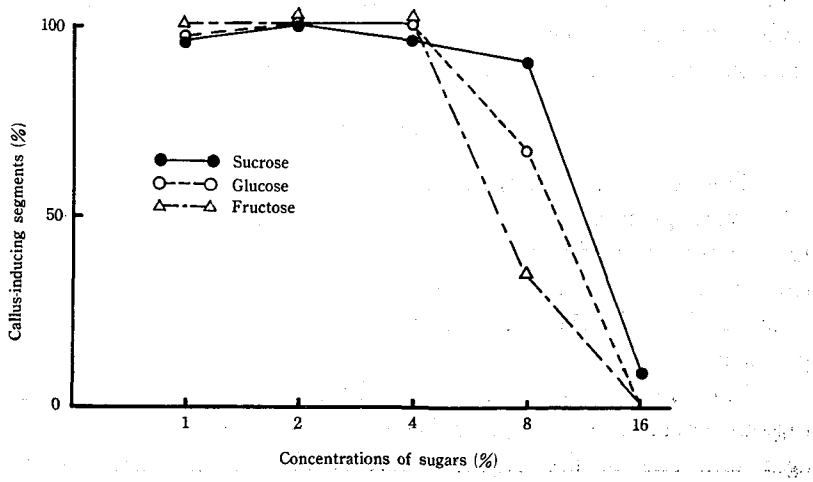

Fig. 1. Effect of sugars on callus induction in the *in vitro* culture of internode segments excised from the shoots of asparagus seedlings (after 16 weeks of culture).

sugar, and showed slight differences among these sugars (Fig. 3). The number of shoots showed a similar tendency with each sugar, while, as to 2% of these sugars, it was highest in glucose, and lowest in sucrose (Fig. 4).

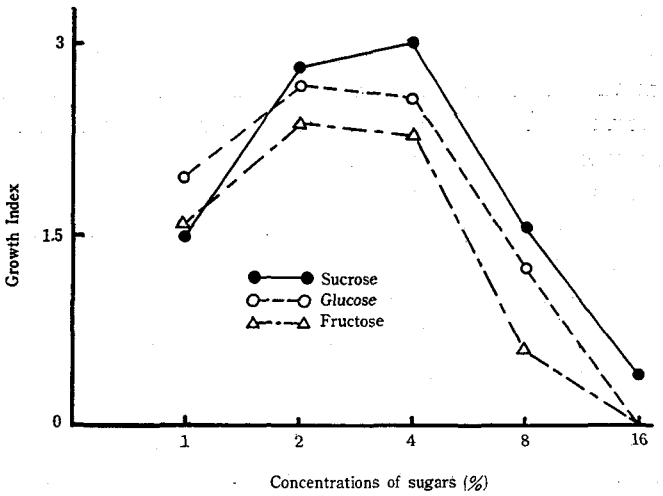

Fig. 2. Effect of sugars on callus growth in the *in vitro* culture of internode segments excised from the shoots of asparagus seedlings (after 16 weeks of culture).

Indices; 1: size as large as the volume of a sphere 3 mm in diameter, 2: 5 mm, 3: 7 mm, 4: 9 mm, respectively.

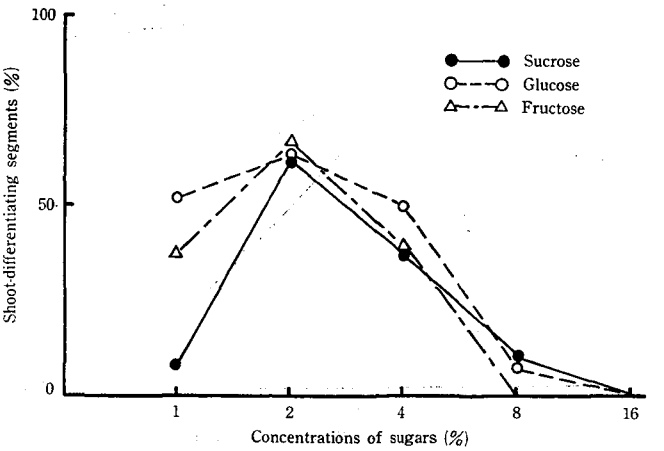

Fig. 3. Effect of sugars on shoot formation in the *in vitro* culture of internode segments excised from the shoots of asparagus seedlings (after 16 weeks of culture).

But, generally the kinds of the sugars did not play a very important role with the shoot formation compared with the concentrations.

Roots began to redifferentiate from the callus after 6 weeks of culture. The percentage of root-redifferentiating callus clumps was highest at 4% of each sugar, and showed but slight differences among the sugars (Fig. 5). The average number of roots per callus clumps was largest at 4% of each sugar, and at the same concentrations, it was largest in sucrose and lowest in fructose respectively (Fig. 6). All roots redifferentiated from the callus in this experiment. Thus, in the case of tissue culture of the shoot segments of asparagus seedlings as in this experiment, the concentrations of sugars appropriate for the root formation were somewhat higher than those for shoot formation.

It was previously reported that various kinds of roots redifferentiated in tissue culture of asparagus.[12] When the concentrations of sugars added to the media were low, the roots redifferentiating from the callus were thin, short, and semitransparent, while at high concentrations of sugars, it developed into thick, long and rigid roots. These roots were vigorous, akin to storage roots and suitable for the formation of intact plants.

This may be related with the fact that the roots of asparagus contain very high concentrations of sugar, and also might be related to the following

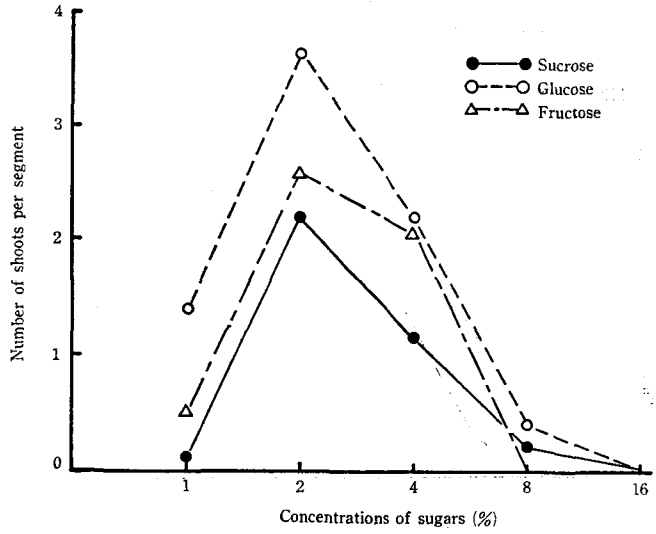

Fig. 4. Effect of sugars on the number of shoot differentiated from callus in the *in vitro* culture of internode segments excised from the shoots of asparagus seedlings (after 16 weeks of culture).

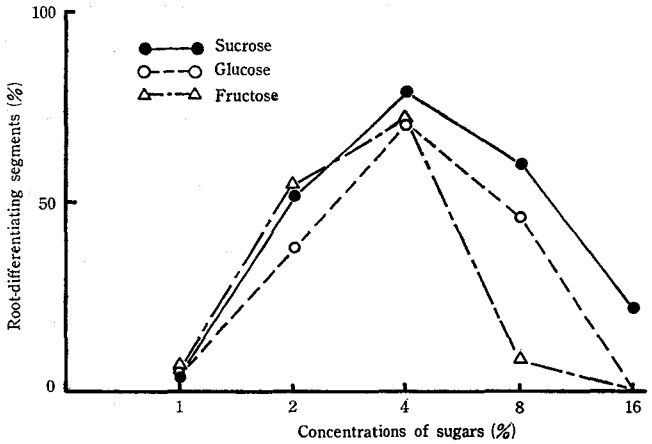

Fig. 5. Effect of sugars on root formation in the *in vitro* culture of internode segments excised from the shoots of asparagus seedlings (after 16 weeks of culture).

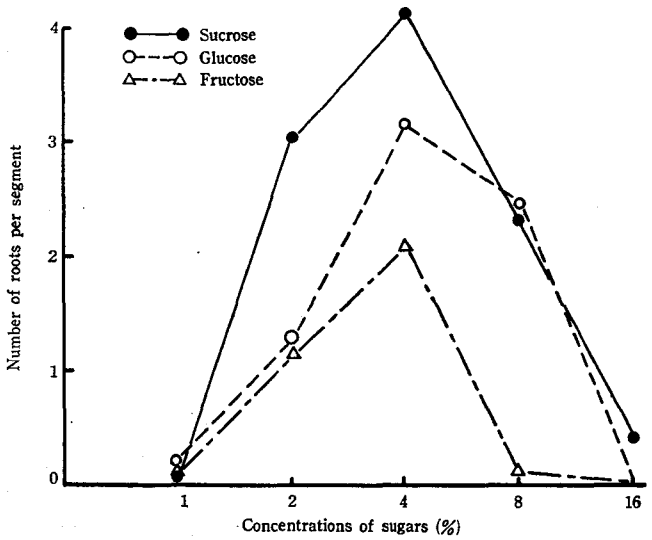

Fig. 6. Effect of sugars on number of roots in the *in vitro* culture of internode segments excised from the shoot of asparagus seedlings (after 16 weeks of culture).

facts: The amount of sugars contained in the storage roots of asparagus varied with the growing season throughout the year. Namely, in a field in a area with clear changes of seasons as in Japan, the amount of the sugar was large from late autumn to early spring, and contribute to the sprouting

of spears in spring. Also, it continues to decrease through harvesting season which is usually from spring to mid summer, and to increase with the translocation of products synthesized in aerial parts from summer to autumn.[11] In addition, the storage roots of asparagus contain various kinds of saccharides.[9,10]

Regarding the above-mentioned facts in the plant level in a field, it is interesting that long, thick and rigid roots akin to a storage root were redifferentiated from the calluses with the addition of high concentrations of sugars in this experiment, and it may be that various saccharides play a certain kind of role in redifferentiation of the organs such as shoots and roots.

Consequently, from the results of this experiment, it was concluded that each of sucrose, glucose and fructose were utilized as a carbon source by the segments excised from the internode of the shoot of asparagus seedlings, and showed the effectiveness on the callus and organ formation.

In recent years, it is said that some saccharides play a very important role in the cell membrane in relation to the differentiation and recognition in morphogenesis. It is desiarable that the further studies on the physiological functions of saccharides will be carried out in connection with the dedifferentiation and redifferentiation in the tissue and cell culture of asparagus.

Summary

To clarify the morphogenesis of asparagus, segments excised from an internode of the first shoot of the seedlings (*Asparagus officinalis* L. cv. Mary Washington 500) were aseptically cultured, and particularly in this experiment, the effect of sugars were examined in relation to callus and organ formation.

The media used contained MURASHIGE and SKOOG's inorganic and organic substances, 1 mg/l of NAA combined with 0.1 mg/l of BA, 7 g/l of agar and one of three kinds of the sugars (sucrose, glucose and fructose) of which concentrations were 0, 1, 2, 4, 8 and 16%. The cultures were maintained at 25°C under 16-hour daily illumination. The results obtained were summarized as follows.

The percentage of callus-forming segments was 90 to 100% at 1 to 4% of the sugars. It decreased with the increase of the concentrations of over 8%, and callus formation was hardly observed at 16% of the sugars. The growth of the callus was good at 2 or 4% of the sugars and not so good at 1, 8, and 16% of these sugars. A slight difference was seen in the callus formation among the sugars.

Percentage of shoot-redifferentiating callus clumps and number of the shoots was largest at 2% of each sugar. In the shoot redifferentiation, the concentrations of the sugars are more important than the kind of sugars.

The percentage of root-redifferentiating callus clumps and the number of the roots were largest at 4% of the sugars, and the concentrations of sugars suitable for rootformation were higher than those for shoot formation. The roots redifferentiated from the callus were thin, short, and semitransparent at low concentrations of the sugars, while it was thick, long, rigid and akin to a storage root at high concentrations of the sugars.

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