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UTILIZATION OF SUGARS AND STARCH AS CARBON SOURCES IN THE ROOTING OF ETIOLATED STEM SEGMENTS OF *POPULUS NIGRA*

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Summary

Stem segments of *Populus nigra* L. did not root in water or auxin alone but did so in 0.5% ribose, glucose and sucrose, sucrose being most effective. The addition of 0.1 mg/l IAA or IBA to the medium stimulated rooting; IBA being more effective. Starch also induced rooting especially in combination with auxins. Starch was hydrolysed by enzymes that leached out of the segments and auxins enhanced their activity.

Introduction

Carbohydrates and auxins influence the ability of stem cuttings to root (Pearce, 1943; Negishi and Satoo, 1956; Sen and Bose, 1958; Hyun, 1967; Nanda, Purohit and Mehrotra, 1968). Auxin effects on rooting are determined by nutritional status and a correct balance of the two is necessary (Nanda and Jain, 1971). This investigation determined the relative effectiveness of some soluble sugars and starch in the rooting of etiolated stem segments.

Materials and methods

Fifteen-cm stem cuttings of *Populus nigra* L., taken from trees growing in the University Campus, were planted in sand in earthenware pots and kept in darkness. The etiolated branches that developed from the axillary buds were cut into 2.5-cm segments.

In the first experiment, 360 stem segments were divided into eighteen samples of twenty segments each. These segments were planted vertically in holes on polythene sheets stretched over Petri dishes (10 cm diameter) containing test solutions as detailed in Table 1. Half the segments in each treatment were kept in continuous light from cool white fluorescent tubes and incandescent filament lamps at an intensity of about 3200 lux and the other half in continuous darkness in an air-conditioned room at $28 \pm 2°$ C. All the test solutions contained chloramphenicol (30 μM) to prevent microbial growth and were changed at 2-day intervals for 20 days. The number of segments rooted and the number of roots were recorded.

In a second experiment, 240 sterilized stem segments were divided into three samples of eighty which were subdivided into four groups of twenty. These were loosely tied in bundles and aseptically (Nanda and Jain, 1971) placed vertically in culture tubes with their basal ends dipping into 25 ml of either (a) water, (b) 1.0 mg/l IAA, (c) 0.125%

starch; or (d) 1.0 mg/l IAA+0.125% starch for 24 hours. The media were analysed after the following treatments.

Step 1. Immediately after removing the segments, 5 ml from treatments (c) and (d) were analysed for starch.

Step 2. After removing the segments, 5 ml 0.25% starch were added to 15-ml aliquots from each treatment and incubated for a further 24 hours. Five-ml aliquots were analysed for starch and two further 5-ml aliquots were treated as follows.

Step 3. Half-ml 0.25% starch was added to both, but one was then boiled. Analyses for starch were made after a further incubation for 24 hours.

Distilled water/starch controls were included for all three steps. Starch was estimated by adding 0.2 ml 0.6% iodine in 6% aqueous potassium iodide and measuring optical density with a Spectronic 20 with a yellow filter. Values were determined from a standard curve. Results are presented as percentage hydrolysis, calculated from initial and residual values.

RESULTS

Rooting of segments in various media (*Expt* 1, *Table* 1)

Roots were not initiated on segments in water or auxins alone but emerged on segments in the sugar solutions. No segments rooted in starch in the light and only a few

Table 1. *Effect of different sugars and starch alone, or together with IAA and IBA, on the number of etiolated stem segments of* Populus nigra (2.5 *cm*) *that rooted, the number of roots per rooted segment and per segment (figures within parentheses) in continuous light or darkness (sample size: ten segments)*

Treatment	No. of segments rooted		No. of roots per rooted segment (per segment)	
	Light	Dark	Light	Dark
Control	0	0	–	–
IAA 0.1 mg/l	0	0	–	–
IBA 0.1 mg/l	0	0	–	–
Ribose 0.5%	2	1	1.0 (0.2±0.1)	1.0 (0.1±0.1)
Glucose 0.5%	6	4	1.2 (0.7±0.2)	1.7 (0.7±0.3)
Sucrose 0.5%	5	5	1.6 (0.8±0.3)	1.6 (0.8±0.3)
Starch 0.5%	0	3	–	1.0 (0.3±0.1)
Starch 1.0%	0	2	–	1.0 (0.2±0.1)
IAA 0.1 mg/l+ribose 0.5%	2	4	1.5 (0.3±0.2)	1.5 (0.6±0.2)
IAA 0.1 mg/l+glucose 0.5%	8	10	2.4 (1.9±0.4)	2.6 (2.6±0.3)
IAA 0.1 mg/l+sucrose 0.5%	6	10	2.2 (1.3±0.4)	2.5 (2.5±0.3)
IAA 0.1 mg/l+starch 0.5%	0	2	–	1.0 (0.2±0.1)
IAA 0.1 mg/l+starch 1.0%	2	6	2.0 (0.4±0.2)	2.6 (1.6±0.4)
IBA 0.1 mg/l+ribose 0.5%	5	5	2.0 (1.0±0.2)	2.5 (1.7±0.6)
IBA 0.1 mg/l+glucose 0.5%	10	10	5.5 (5.5±0.5)	5.0 (5.0±0.6)
IBA 0.1 mg/l+sucrose 0.5%	10	10	7.2 (7.2±0.9)	8.6 (8.6±0.1)
IBA 0.1 mg/l+starch 0.5%	9	4	6.0 (5.4±0.4)	2.5 (1.4±0.1)
IBA 0.1 mg/l+starch 1.0%	10	10	10.4 (10.4±0.6)	8.0 (8.0±0.4)

in darkness. The number of rooted segments and roots were similar in glucose and sucrose but lower in ribose. Auxins added to sugar or starch media increased the number of rooted segments, IBA being more effective than IAA in both light and dark. The number of roots also increased with auxins in the medium, IBA producing more in all cases. This effect was most pronounced with 1.0% starch and 0.5 sucrose, less so with glucose and least with ribose. With 0.5% starch it was markedly higher in light than in darkness.

Utilization of starch during rooting (*Expt* 2)

The rooting of stem segments in starch suggested that either starch-hydrolysing enzymes leached out of segments or starch molecules were absorbed intact.

The activity of hydrolysing enzymes in solutions of starch alone or in combination with IAA was such that 99% of the starch supplied was hydrolysed within 24 hours. In controls, starch remained unchanged (step 1).

Table 2 shows the amount of starch left in 5-ml aliquots of different solutions to which

Table 2. *Hydrolysis of starch by 5-ml samples of leachates from etiolated stem segments of* Populus nigra *into different test solutions* (*for details see Materials and methods*)

Solution	Initial starch (mg/sample)	Starch left after 24 h (mg/sample)	% starch hydrolysed
Control	3.12	3.12	0
Water	3.12	2.52	19
IAA 1.0 mg/l	3.12	1.74	44
Starch 0.125%	3.21	0.72	77
Starch 0.125% + IAA 1.0 mg/l	3.21	0.48	85

5 ml of 0.25% starch was added (step 2). The activity of hydrolysing enzymes was least in treatment with water and progressively greater with IAA, with starch, and with starch + IAA.

Table 3 shows that hydrolysis of starch in culture tubes containing boiled leachates was markedly less than those with unboiled starch (step 3). In the unboiled samples the

Table 3. *Hydrolysis of starch by leachates from etiolated stem segments of* Populus nigra *to boiled and unboiled 5.5-ml samples of different test solutions* (*for details see Materials and methods*)

Solution	Initial starch (mg/sample)	Starch left after 24 h (mg/sample)		% starch hydrolysed	
		Unboiled	Boiled	Unboiled	Boiled
Control	1.32	1.32	1.32	0	0
Water	3.84	1.74	3.60	54	6
IAA 1.0 mg/l	3.06	1.26	2.98	58	2
Starch 0.125%	2.04	0.72	1.84	86	9
Starch 0.125% + IAA 1.0 mg/l	1.80	0.60	1.66	70	9

hydrolytic activity was more with leachate into starch alone and into starch together with IAA than those lacking starch.

Discussion

The results demonstrate that rooting of etiolated stem segments of *Populus nigra* is limited primarily by nutritional factors, as segments that failed to root in water or auxin alone rooted in sugar solutions. Stem segments can also use starch as a carbon source and its utilization in rooting is effected by hydrolytic enzymes which leach into the test solution. Nickell and Maretzki (1970) considered that the utilization of starch by sugar-cane cells was due to secretion of α-amylase. The secretion of α-amylase by *Rumex* virus tumors *in vitro* and also of an enzyme from intact cells of *R. acetosella* were also reported earlier by Brakke and Nickell (1951, 1952, 1955).

The enhanced rooting in starch + auxin is due to enhanced activity of hydrolysing

enzymes which mobilize the starch into soluble sugar to a level that balanced correctly with the concentration of exogenously applied auxins. The role of auxins therefore is two-fold: firstly to mobilize reserve food materials; and secondly to enhance cell division, elongation and differentiation. That auxins are able to mobilize reserve food materials by enhancing the activity of hydrolysing enzymes has been demonstrated earlier (Wort and Cowie, 1953; Nanda and Anand, 1970).

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