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INTERACTION EFFECTS OF GLUCOSE AND AUXINS IN ROOTING ETIOLATED STEM SEGMENTS OF *SALIX TETRASPERMA*

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SUMMARY

Etiolated, 2.5 cm long stem segments of *Salix tetrasperma* did not root when cultured in agar even with 0.1% glucose, but rooted with 1.0% glucose in the medium. Rooting decreased with 2.0% and was completely inhibited by 5.0% glucose. Both IAA and IBA induced rooting when added to 0.1% glucose, the number of rooted segments decreasing with the concentration of IAA but increasing with that of IBA. Both auxins increased the number of roots when added to 1.0 and 2.0% glucose, the effect increasing with concentration except that 5.0 mg/l IBA lowered the number as compared to 1.0 mg/l added to 1.0% glucose. Auxins also induced rooting when added to 5.0% glucose, which alone inhibited it completely. The effect of auxins on the number of roots, however, decreased with the increasing concentration of glucose in the medium except with 5.0 mg/l IAA which increased it. The results show that auxin effects on rooting are influenced by nutritional status of stem cuttings and that a proper balance of the two is necessary for this purpose.

INTRODUCTION

Stem cuttings of plant species vary considerably in their ability to root and some do not root at all even with auxin application. Excellent reviews on the factors that influence rooting are available (Allen and McComb, 1955; Niensteadt *et al.*, 1958; Dore, 1965). The effectiveness of auxins in rooting stem cuttings varies with season (Nanda *et al.*, 1968; Nanda and Anand, 1970). The present investigations used small segments from etiolated branches emerging from stem cuttings of a number of plants to study their rooting response to nutrients, regulatory substances and environmental conditions. The interaction of glucose and auxins on rooting etiolated stem segments of *Salix tetrasperma* are reported in this paper.

MATERIALS AND METHODS

Stem cuttings, 15 cm long, of *S. tetrasperma* from a tree growing in the University Campus were planted in sand in earthenware pots in the dark. The etiolated branches that developed from the axillary buds were cut into 2.5 cm segments, sterilized by immersing in 0.1% solution of mercuric chloride for 5 minutes and washed thoroughly with sterilized distilled water. A total of 350 segments was divided into thirty-five groups of ten segments. Segments were transferred, two in each culture tube (diameter 2.5 cm) containing sterilized agar medium (0.9%) with or without glucose and auxins in an inoculation chamber previously sterilized with ultraviolet light. Five culture tubes were used for each treatment and these were kept in continuous dark in an air-conditioned room at a temperature of 26 ± 1° C.

Observations on the number of segments that rooted and the number of primary and secondary roots produced were recorded after 20 days. The estimates of callus produced were also made by giving arbitrary score numbers.

RESULTS

Number of rooted segments and roots

The results in Tables 1 and 2 show that segments did not root on agar alone or with 0.1% glucose but rooted with 1.0% glucose. The number of rooted segments and roots

Table 1. *Effect of different concentrations of glucose alone or together with varying concentrations of IAA and IBA on the number of etiolated* Salix tetrasperma *segments (2.5 cm long) that rooted out of 10 cultured in agar medium in darkness*

Concentration of glucose (%)		Concentration of auxin (mg/l) IAA			IBA		
	0	0.1	1.0	5.0	0.1	1.0	5.0
0	0	0	1	0	1	4	0
0.1	0	6	2	1	2	4	0
1.0	8	8	8	10	10	10	8
2.0	3	7	8	7	9	9	9
5.0	0	7	7	7	8	7	10

Table 2. *Effect of different concentrations of glucose alone or together with concentrations of IAA and IBA on the number of roots per rooted segment and per segment (figures within parentheses) produced on etiolated* Salix tetrasperma *segments (2.5 cm long) cultured in agar medium darkness.*

Concentration of glucose (%)		Concentration of auxin (mg/l) IAA			IBA		
	0	0.1	1.0	5.0	0.1	1.0	5.0
0	–	–	1.0 ± 0.0 (0.1 ± 0.1)	–	1.0 ± 0.0 (0.1 ± 0.1)	2.0 ± 0.5 (0.8 ± 0.4)	–
0.1	–	5.1 ± 0.9 (3.2 ± 0.9)	2.0 ± 0.0 (0.4 ± 0.2)	2.0 ± 0.0 (0.2 ± 0.2)	4.5 ± 0.5 (0.9 ± 0.4)	4.5 ± 0.6 (1.8 ± 0.7)	–
1.0	2.2 ± 0.5 (1.8 ± 0.3)	7.5 ± 2.1 (6.1 ± 2.0)	8.7 ± 0.3 (7.0 ± 0.3)	8.8 ± 0.9 (8.8 ± 0.9)	5.2 ± 0.5 (5.2 ± 0.5)	15.7 ± 2.1 (15.7 ± 2.1)	13.3 ± 0.4 (10.7 ± 2.1)
2.0	1.0 ± 0.0 (0.3 ± 0.1)	4.4 ± 1.6 (3.1 ± 1.0)	6.0 ± 1.1 (4.7 ± 1.3)	9.7 ± 2.0 (6.8 ± 1.6)	4.3 ± 0.8 (4.0 ± 0.8)	9.3 ± 0.9 (8.5 ± 1.2)	9.8 ± 0.9 (8.9 ± 1.2)
5.0	–	4.0 ± 0.9 (2.8 ± 0.9)	4.4 ± 1.2 (3.1 ± 1.0)	10.1 ± 1.4 (7.1 ± 1.5)	4.8 ± 0.6 (3.9 ± 0.7)	5.7 ± 1.8 (4.0 ± 1.2)	8.5 ± 1.0 (8.5 ± 1.0)

decreased with 2.0% and was completely inhibited by 5.0% glucose. IAA alone did not initiate roots except 1.0 mg/l which induced one root on one segment only. It is interesting that while rooting did not occur with either 0.1% glucose or 0.1 mg/l IAA alone, six out of ten segments rooted with 5.1 roots on each when the two were used together. The number of both rooted segments and roots, however, decreased with higher concentrations of IAA. 0.1 and 1.0 mg/l IBA alone or together with 0.1% glucose also induced rooting in a few segments while 5.0 mg/l IBA inhibited it completely.

Rooting occurred on eight segments with 1.0% glucose and the number of roots increased with the addition of 0.1 and 1.0 mg/l of IAA or IBA. It did not increase above

1.0 mg/l with 5 mg/l IAA but decreased slightly with 5.0 mg/l IBA. However, with 2.0% glucose the number of roots increased with the increasing concentrations of both IAA and IBA. Rooting was caused even with 5.0% glucose when IAA or IBA was added and the number of roots increased with the concentration of both auxins. The effect of 5.0 mg/l IBA decreased while that of 5.0 mg/l IAA increased with the increasing concentration of glucose.

Secondary roots

Segments cultured on 1.0% glucose together with 0.1 and 1.0 mg/l IBA produced secondary roots but not with 5.0 mg/l IBA, although the primary roots with this concentration of IBA were much swollen. The number of secondary roots increased with 2.0% and again with 5.0% glucose and also increased with the increasing concentration of IBA added to a given concentration of glucose.

Callus formation

The amount of callus produced on segments is presented as score numbers in Table 3.

Table 3. *Effects of different concentrations of glucose alone or together with varying concentrations of IAA and IBA on the degree of callus that produced on etiolated* Salix tetrasperma *segments (2.5 cm long) cultured in agar medium in darkness*

Concentration of glucose (%)	Concentration of auxin (mg/l)						
		IAA			IBA		
	0	0.1	1.0	5.0	0.1	1.0	5.0
0	0	0	1	0	0	2	3
0.1	0	3	3	8	3	5	8
1.0	0	6	10	8	6	15	15
2.0	0	8	15	15	5	18	22
5.0	0	5	10	15	5	15	28

Segments cultured with auxins in the medium produced callus, particularly in the presence of glucose. The callus formation was observed as a swelling at the base after 7 days followed soon by a rupture of the periderm. The growth became conspicuous after 12 days and the magnitude of callus, in general, increased with an increase in the concentration of auxin added to a given concentration of glucose. IBA was more effective than IAA and the effect did not differ with 1.0 and 5.0 mg/l IAA added to 2.0% glucose but was higher with 5.0 mg/l auxins added to 5.0% glucose. It was observed that the callus mass was localized to the basal end of segments cultured with IAA but spread all over its surface with IBA in the medium and that the adventitious roots emerged at the time when callus ruptured the periderm. The roots that emerged also were localized at the end in IAA-treated but were spread all over the surface in IBA-treated segments.

Discussion

The results demonstrate that the rooting of small etiolated segments of *Salix tetrasperma* is limited primarily by nutritional factors. Thus, rooting did not occur in agar even with 0.1% but occurred with 1.0% glucose in the medium. The decrease in the number of rooted segments and roots with higher concentrations of glucose may be due to high osmotic pressure causing plasmolytic changes. The beneficial effect of carbohydrates on rooting has been reported by other workers also (Negishi and Satoo, 1956; Went and Thimann, 1937; Pearce, 1943; Preston, Shanks and Cornell, 1953; Sen and Bose, 1958; Hyun, 1967).

Rooting was enhanced by IAA and IBA added to glucose. A few segments rooted even with 1.0 mg/l IBA alone. This may be ascribed to its effect of increasing the activity of hydrolytic enzymes so making reserve food available for division and differentiation of cells into roots. (See Nanda and Anand, 1970; Bala, Anand and Nanda, 1970.)

The most significant point that emerges from this investigation is the interaction of auxins with nutritional factors. Thus, with 0.1% glucose rooting was maximum with 0.1 mg/l IAA or IBA; it decreased when the concentration of auxin was increased to 1.0 mg/l and was completely inhibited when auxin concentration was increased to 5.0 mg/l. With 1.0% glucose the most effective concentration of auxin was 1.0 mg/l, 5.0 mg/l IBA being inhibitory. The effectiveness of auxins in rooting segments was lower with 2.0 or 5.0% than with 1.0% glucose except 5.0 mg/l IAA in which the number of roots increased with the concentration of glucose.

The nature of the auxin also determines the rooting response of segments. Thus, the increase in number of roots with the addition of IBA to 1.0% glucose was more than with IAA. Secondary roots were also produced with IBA and not with IAA. The magnitude and distribution of callus also varied with the auxin. Thus, 0.1 and 1.0 mg/l IAA produced the maximum amount of callus with 2.0%, but 5 mg/l IAA produced it with 5.0% glucose. In contrast to this 0.1 mg/l IBA produced maximum callus with 1.0%, 1.0 mg/l with 2.0% and 5.0 mg/l with 5.0% glucose. Again while with IAA callus was confined to the basal end it was distributed all over the length of the segment with IBA. This difference in the effect of two auxins may be related partly to the differential rate of polar transport of the two auxins and partly to the fact that while IAA oxidase brings about the oxidation of IAA, the effect of IBA is persistent, being a strong auxin (unpublished data). It may, therefore, be concluded that the ability of stem cuttings to root is determined by a proper balance between nutrition and regulatory factors and that rooting may not occur even when the concentration of one is very high.

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