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REGENERATION OF *TARAXACUM* ROOTS IN RELATION TO CARBON AND NITROGEN SUPPLY.*

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Abstract

Regeneration of *Taraxacum officinale* Weber., root cuttings in relation to carbon and nitrogen supply was studied. It was found that the application of KNO_3 promoted the growth of shoots and roots. Sucrose alone, promoted the root growth only slightly whereas sucrose and KNO_3 showed a pronounced effect on root growth. Organ differentiation in undifferentiated *Taraxacum* root callus did not occur when sucrose, KNO_3 or both was provided. However, roots and shoots were induced in the fresh phoem explants by altering the level of auxin (IAA) and cytokinin (Adenine) and the particular organ developed was dependent on the relative concentration of these hormones.

Introduction

Kraus & Kraybill (1918) working with the tomato plant, developed a theory that the behaviour of the plant, as regards vegetative growth and sexual reproduction, depends to a large extent upon the relative proportion of the available carbohydrates and nitrogen within the plant body. Reid (1924) also studied the role of carbohydrate and nitrogen in regenerating tomato stem cuttings and found that when the carbohydrate reserves were high and nitrogen low within the cuttings, vigorous root growth resulted. However when the relative percentage of nitrogen was slightly higher, vigorous shoot growth was found.

An attempt has been made to elucidate whether the regeneration of root cuttings could be modified by supplying carbhoydrate and nitrogen to them externally. Satchuthananthavale (1966) has established callus cultures of *Taraxacum* roots and has shown that the regenreration can be controlled by adjustment of the auxin and cytokinin levels in the culture medium. It seemed relevant to test here the effect of varying carbohydrate and nitrogen supply on the regeneration of this callus isolates and the fresh phloem explants.

Materials and Methods

Roots of *Taraxacum officinale* Weber, were collected from the open ground in the vicinity of Sheffield University and brought to the laboratory in polythene bags.

Supply of carbohydrate and nitrogen to the root segment.

Whole roots were dipped in 0.1% $HgCl_2$ solution for 3 min and washed with sterile water. After marking the proximal (shoot-end of the plant) and distal (root-end of the plant) ends with red ink, 2 cm long segments were excised in a sterile cham-

*This work was carried out at the department of Botany, University of Sheffield, Sheffield, England.

ber and the segments were left in sterile distilled water until required. The cut ends of the surface-dried root cuttings were placed in contact with agar, with or without sucrose or KNO_3. Sterile 0.8% agar, alone and with 4% sucrose or KNO3 was prepared and used to fill 9 cm Petri dishes (20 ml) or to make agar rods. The latter were made by filling sterilized 1.25 cm diameter glass tubes, with one end closed with non-absorbant cotton wool. After cooling, the solidified agar was pushed out as an agar rod.

For treatment of one end only, root segments were kept vertically with the desired end touching the agar kept in the petri dishes. For treatment of both ends of the cuttings, two agar rods were kept horizontally, parallel to each other, on two seperate microscopic slides held 0.5 cm apart in 9 cm Petri dishes. The root segments were then placed horizontally with each end touching the agar rods. The Petri dishes were covered with 12 cm diameter petri dishes and transfered to plastic lunch boxes kept at 25°C in the dark. Thirty root segments were used for each treatment. After 24 hr. of treatment, root segments were taken out washed thoroughly with water and left horizontally 1 cm beneath the sand-vermiculite mixture (5:1) at 5°C in the dark and watered every day. After six weeks, number, length and dry weight of shoots and roots were recorded.

Callus culture

Nutrient solutions were prepared according to White (1943) and supplemented with 15% coconut milk since Booth & Satchuthananthavale (1974-b) found a significant increase in the growth of *Taraxacum* root callus. Sucrose and KNO_3 supplements were added to the basal medium to give two series of treatments, viz., 0.008% KNO_3 with 2, 4, 6 or 8% sucrose and 2% sucrose with 0.008, 0.016, 0.024 or 0.032% KNO_3 in 0.8% Oxoid agar. The media were dispersed as 50 ml aliquots in 150 ml conical flasks which were then plugged with non-absorbant cotton and autoclaved at 15 lbs/sq inch pressure for 15 min. Undifferentiated root callus grown in the basal medium plus 0.4 mg/l IAA (stock culture) was inoculated into five flasks for each treatment. The flasks were then incubated at 25°c in the dark and examined every two weeks and finally photographed after ten weeks.

Tissue culture

Phloem pieces (approx. 5 mm^3) of surface sterilized roots were excised from a single root under sterile conditions and inoculated onto agar medium containing auxin and adenine. The following additions were made to the basal medium. Zero (Control); 1 mg/l IAA, 10 mg/l IAA; 1 mg/l IAA plus 20 mg/l adenine; 10 mg/l IAA plus 20 mg/l adenine; 20 mg/l adenine alone.

10 ml aliquots of these solutions were dispersed in 25 ml vials and sterilized by autoclaving at 15 lb/sq inch pressure for 15 min. After colling, one phloem piece was inoculated in each vial and the cap was screwed lightly. The tubes, 10 per treatment, were kept in dark at 25°c for six weeks and photographed.

Results

Effect of carbohydrate and nitrogen supply on the regenerating root segments.

Comparing the results of the treatments with control (Table-1), it was found that sucrose, applied distally or proximally, had little effect, although there is some

indication of reduced leaf number (in case of distal application only) and lesser total lengths of the leaves. Supplying the nitrate enhanced the regeneration of leaves and roots. Distal applications produced higher number of shoots and roots while the proximal application produced fewer but longer ones. The application of sucrose proximally, in addition to nitrate applied distally, gave a slight reduction in organ numbers but increased their length, the reverse being true when sucrose was applied distally and nitrate proximally.

The results of shoots and roots regenerated as a percentage of dry weight of the whole cuttings is presented in Fig. 1. Sucrose suppressed shoot weight when applied at either end but had little effect on roots. Nitrate alone increased shoot weight with its proximal application. Distal application of sucrose, with nitrate at the proximal end, led to the enhancement of root weight. With sucrose at the proximal end and nitrate at the distal end both shoots and in particular roots, weight were increased.

TABLE 1. Effect of sucrose and potassium nitrate supply on the regeneration of *Taraxacum* root segments.

	TREATMENTS						
	A	B	C	D	E	F	G
Number of leaves	2.0	1.4	2.0	4.0	3.7	3.2	4.4
St. error	±0.26	±0.10	±0.18	±0.36	±0.29	±0.19	±0.88
Number of roots	1.6	1.5	2.0	5.0	2.6	4.6	3.5
St. error	±0.21	±0.23	±0.10	±0.48	±0.28	—0.49	±0.75
Total length of leaves	3.4	3.3	5.9	9.8	14.0	14.5	13.8
St. error	±0.88	±0.69	±0.98	±0.62	±2.38	±3.80	±2.68
Total length of roots	1.2	1.4	1.8	2.8	8.0	7.2	6.1
St. error	±0.18	±0.09	±0.14	±0.25	±1.98	±2.64	±1.80

A — Control
B — 4% sucrose applied at the distal end;
C — 4% sucrose applied at the proximal end;
D — 2% KNO3 applied at the distal end;
E — 2% KNO3 applied at the proximal end;
F — 2% KNO3 applied at the distal end and 4% sucrose applied at the proximal end;
G — 4% sucrose appled at the distal end and 2% KNO3 applied at the proximal end.

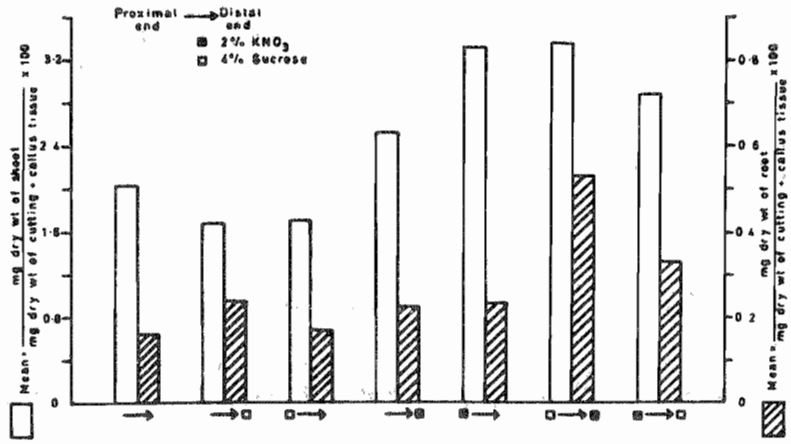

Fig. 1. Effect of sucrose and KNO3 supply form proximal and distal ends of the *Taraxacum* root cuttings on the dry weight of shoots and roots

Callus culture

Increasing concentration of sucrose with a fixed nitrate level were found to have no effect in terms of shoot and root initiation (Fig. 2). Better callus growth

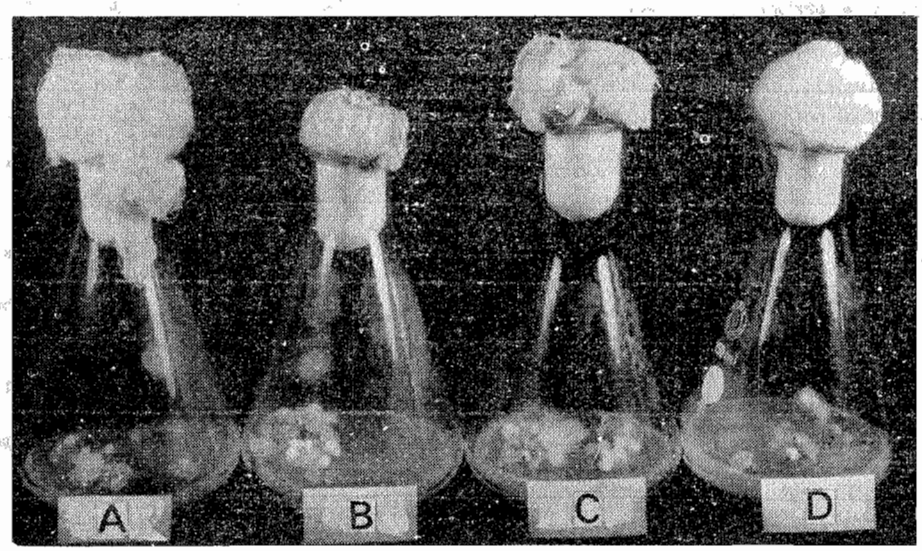

Fig. 2. Effect of different concentrations of sucrose on growth and organ formation in *Taraxacum* root callus grown on White's medium for a period of ten weeks.

A—2% sucrose — 0.008% KNO_3 (Control);

B—4% sucrose — 0.008% KNO_3;

C—6% sucrose — 0.008% KNO_3;

D—8% sucrose — 0.008% KNO_3;

was noted with 4 and 6% sucrose. When nitrate was increased it also showed no effect on shoot or root initiation at any concentration (Fig. 3). However, vigorous callus growth was found in all the three nitrate treatments, especially at 0.016% KNO_3

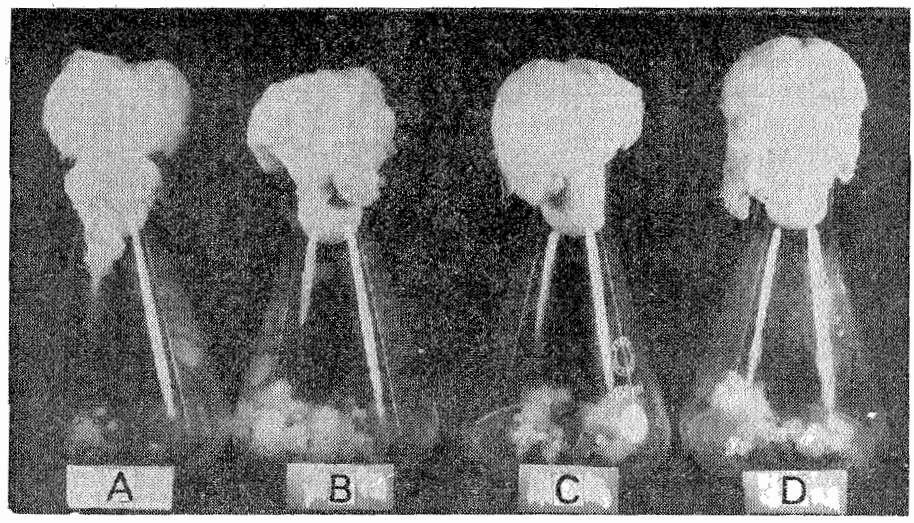

Fig. 3. Effect of different concentrations of potassium nitrate on growth and organ formation in *Taraxacum* root callus growfl on White's mediuj for a period of tefl weeks.
A - 2% Sucrose - 0.008% KNO_3:
B - 2% Sucrose - 0.016% KNO_3·
C - 2% Sucrose - 0.024% KNO_3,
D - 2% Sucrose - 0.032% KNO_3.

Tissue culture

Effect of IAA, adenine and both on the initiation of shoots and roots from phloem explants of *Taraxacum* roots are presented in Table-2 and Fig-4. It was found that in the controls neither shoots nor roots were formed and very little callus growth was noted. With 1 mg/1 IAA shoots were initiated whereas at 10 mg/1 root initiation occured. On the other hand 20 mg/1 adenine promoted shoot regeneration only. A marked interaction between IAA and adenine was found in terms of shoot and root initiation. Not only did the addition of adenine to 1 mg/1 IAA increased shoot initiation but also roots were formed. Similarly 10 mg/1 IAA, which alone gave roots, with adenine gave shoots and roots.

Discussion

The present study has revealed that the external application of KNO_3 at the proximal and distal cut ends enhanced the growth of shoots and roots in the regenerating *Taraxacum* root cuttings whereas sucrose produced no significant effect. Vigorous root growth resulted with the application of both KNO_3 and sucrose. Hicks (1928),

TABLE 2. Effect of Indoleacetic acid and adenine on the regeneration of phloem explants of *Taraxacum* roots. (Each value is the mean of ten replicates.)

Treatment	No. of leaves per segment	Total length of leaves per segment (cm)	No. of roots per segment	Total length of roots per segment (cm)
Control	0.0	0.0	0.0	0.0
1 mg/l IAA	1.5	2.4	0.0	0.0
1 mg/l IAA + 20 mg/l Adenine	4.0	9.7	2.0	1.3
10 mg/l IAA	0.0	0.0	6.0	14.2
10 mg/l IAA + 20 mg/l Adenine	1.0	3.8	5.0	13.5
20 mg/l Adenine	5.0	10.1	0.0	0.0

while studying the chemistry of growth as represented by carbon-nitrogen ratio in *Salix viminalis* stem cuttings, reported a basipetal translocation of carbohydrates. However, nitrogenous compounds moved acropetally. Due to this differential

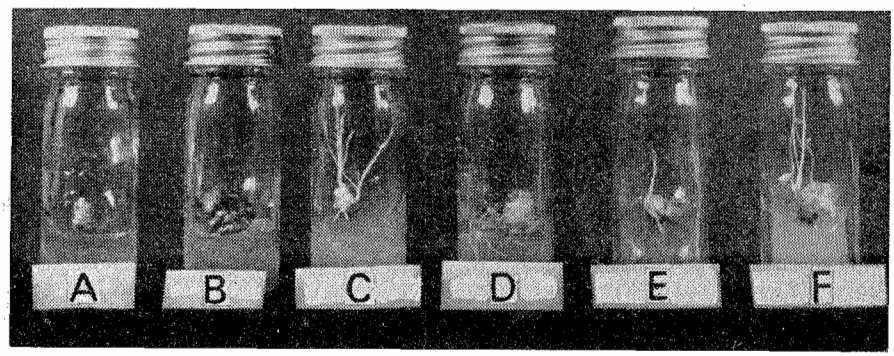

Fig. 4. Effect of IAA, Adenine and both on the initiation and development of shoots and roots from phloem explants of *Tarasacum* roots.
A —Control;
B—1 mg/l IAA
C—1 mg/l IAA + 20 mg/l Adenine;
D —10 mg/l IAA;
E - 10 mg/l IAA + 20 mg/l Adenine·
F 20 mg/l Adenine.

translocation, the carbon-nitrogen ration was found to be high at the base, where the roots developed, than at the apex where shoots develop. Similar results were obtained by Davis (1931) who observed root developing in regions where nitrogen, as percentage of dry material, was low and shoots where it was high in *Salix* stem cuttings.

It appears from the present study that the effect of sucrose and KNO_3 on organ formation may not be their direct effect since *Taraxacum* root cuttings contains enough endogenous anxins and cytorinins which are perhaps responsible for the hormonal control of regeneration in *Taraxacum* roots (Booth & Satchuthanathavale, 1974-a). The use of unorganised *Taraxacum* root callus provided an opportunity for testing the direct effect of these nutrients on organ formation. Neither sucrose nor KNO_3 proved to have any significant effect, when present alone, on the organ formation of root callus cultures although they did have an effect in promoting callus growth. However, Booth & Satchuthananthavale (1974-b) using the same callus isolate, found shoot developing at low auxin concentration (0·1mgl^{-1} IAA) while roots developed at high concentrations (1—10 mgl^{-1} IAA). The present study has also revealed that the organ formation could be induced in fresh phloem explants of *Taraxacum* roots by altering the auxin and cytokinin levels and that the particular type of organ developed was dependent on the relative concentration of these two hormones. Thus it is likely that the effect of carbhohydrate and nitrogen on organ formation, noted by earlier workers as well as in the present study, was not due to their direct effects but to their intereaction with the endogenous hormones.

Acknowledgements

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