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THE POLARITY OF AUXIN TRANSPORT IN INVERTED CUTTINGS

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Summary

The original, basipetal polarity of auxin transport persisted in the stems of inverted cuttings of *Tagetes*, tomato and tobacco in spite of the reversal of the relative positions of the roots and shoots. No significant acropetal auxin transport could be detected even after four months growth. These results indicate that the polarity of newly formed cells in secondarily thickening internodes is determined by the existing polarity of auxin transport within the tissues.

Introduction

The maintenance of the polar auxin transport system depends on auxin transport itself. Tissues of decapitated plants (Leopold and Lam, 1962) or excised shoot tissues (Osborne, Horton and Black, 1968; Veen, 1969) show a decline in their ability to transport auxin basipetally which can be prevented, or at least retarded, by the application of auxin to their apical ends. Sachs (1968, 1969) has shown that the pattern of development of auxin-induced vascular strands in stem tissues depends on the polarity of the tissue, which is maintained if auxin is supplied at the apical end and declines if it is not. If the normal polarity of auxin transport declines or is disrupted, new pathways of auxin movement can develop and induce the formation of new vascular strands, associated with which is a new polarity of auxin transport (Sachs, 1969; Kirschner, Sachs and Fahn, 1971). There is an element of autocatalysis in this system: the transport of auxin maintains the polarity of differentiating vascular cells or cells closely associated with them in such a way that more auxin is transported with the same polarity, leading to more vascular differentiation.

Went (1941) found that in inverted *Tagetes* cuttings which had been induced to form roots at the originally apical end of the stem and which had formed shoots at the originally basal end, the original basipetal polarity of auxin transport in the stem persisted but after about 3 weeks the acropetal movement of auxin could also be detected. More acropetal auxin movement was found in plants which had been grown in an inverted position for longer periods. He concluded that a new polarity had been established in cells associated with newly formed vascular tissues. A reversal of polarity in newly formed cells in spite of the persistence of the original basipetal polarity in the older tissues would imply that the polarity of these cells was determined not by the already established polarity but by physiological gradients dependent on the relative positions of the roots and shoots. Went's results therefore appear to cast serious doubt on the general validity of Sach's (1968, 1969, 1970) findings that the polarity of cells associated with differentiating vascular tissue depends on the existing polarity of auxin transport in

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their vicinity, and that a new polarity of auxin transport can develop only when the original polarity has been lost.

I have re-investigated the polarity of auxin transport in inverted cuttings of *Tagetes* using lower concentrations of auxin, which are considered to be more comparable with those typically found in plant tissues. I was unable to confirm Went's results either with *Tagetes* or with inverted cuttings of other species.

MATERIALS AND METHODS

Plants of *Tagetes patula* L. and tobacco (*Nicotiana tabacum* L. cv. java) were grown from seed in glasshouses at the University Botanic Garden. These were used for experiments when they were about 15 cm and 50 cm high respectively. Young plants of tomato (*Lycopersicon esculenteum* Mill. cv. moneycross) and *Tagetes erecta* L. were purchased locally and were used for experiments when they were about 20 cm high.

Cuttings made from the plants were treated with a commercial rooting powder ('Seradix- No. 1', May and Baker Ltd) containing indol-3yl-butyric acid. The apical end of the cutting was treated with rooting powder and planted in vermiculite; control cuttings were induced to root at the basal end of the stem and were planted in a normal orientation. Axillary buds were removed from the morphologically apical parts of the inverted cuttings. Some plants were grown in vermiculite for the whole period of the experiment, others were transplanted after 3 weeks to soil in plant pots. Plants were grown in glasshouses in winter; in experiments conducted in the summer the plants were grown outdoors. They were watered with tap water supplemented with a commercial 'liquid manure' ('Baby Bio', Pan Britannic Industries Ltd).

For auxin transport measurements, 1 cm segments were excised from the internodes. Segments from inverted cuttings were taken from the part of the stem between the roots and the shoots. Two segments were used for each measurement unless otherwise stated. Donor and receiver agar (1% w/v) blocks were applied; the donor blocks contained 3.0 μM [1-^{14}C]indol-3yl-acetic acid (IAA) (Amersham, 52 mCi/mM). The transport assemblies were set up and the radioactivity in the receiver blocks was counted as described by Sheldrake (1973a). During the transport period the segments were kept in the dark at 22° C.

RESULTS

Cuttings from *Tagetes patula* plants were induced to root by placing the apical ends in a solution of IAA (100 mg/l) for 18 h before being planted in sand; this procedure was the same as that of Went (1941). Segments from the part of the stem between the roots at the apical end and the shoots at the apical end were tested for their ability to transport auxin.

Table 1. *Auxin movement through internode segments from inverted* Tagetes patula *plants induced to root by treatment with a solution of IAA (transport time = 3.5 h)*

Days after inversion	ct/min in receivers			
	Apical segments		Basal segments	
	Acropetal	Basipetal	Acropetal	Basipetal
14	140	193	3	230
18	221	424	2	259
20	143	322	5	90
23	39	59	4	93

Table 2. *Auxin movement through internode segments from inverted and control cuttings of* Tagetes erecta, *tomato and tobacco* (*transport time* = 4 *h*)

(a) *Tagetes*			(b) Tomato			(c) Tobacco		
Age of cuttings (days)	ct/min in receivers		Age of cuttings (days)	ct/min in receivers		Age of cuttings (days)	ct/min in receivers	
	Acropetal	Basipetal		Acropetal	Basipetal		Acropetal	Basipetal
Inverted			Inverted			Inverted		
18	56	1998	30	29	278	60	13	6008
22	12	1543	62	60	1153	88	8	1818
38	10	532	88	5	1734	120	10	1940
40	18	146	120	25	213	120	3	3884
60	8	379	120	6	208			
63	6	854	120	5	294			
			120	89	699			
Controls			Controls			Controls		
22	9	119	30	33	310	88	12	2445
36	12	216	62	9	860			
60	9	196	62	93	1834			
			88	23	898			
			120	4	625			

Considerable acropetal auxin movement was found in segments from the apical parts of the stem, but not in more basal segments. Some representative results are shown in Table 1. The apical segments looked unhealthy and in most of them the pith had degenerated.

In subsequent experiments, *Tagetes* cuttings were induced to root by treatment with indole butyric acid rooting powder. These plants grew better than those treated with IAA solutions. The shoots that developed from axillary buds at the inverted cuttings ultimately flowered. Inverted plants, however, never developed as well as control cuttings planted right way up.

The transport of auxin by segments taken from the stems of these cuttings at periods of up to 64 days after inversion was predominantly basipetal. Some acropetal movement of radioactivity was detected, but little or no more than in segments taken from control (Table 2a). In three separate series of experiments, one with *T. patula* and two with *T. erecta*, no evidence was obtained for any pronounced acropetal auxin movement such as that described by Went (1941).

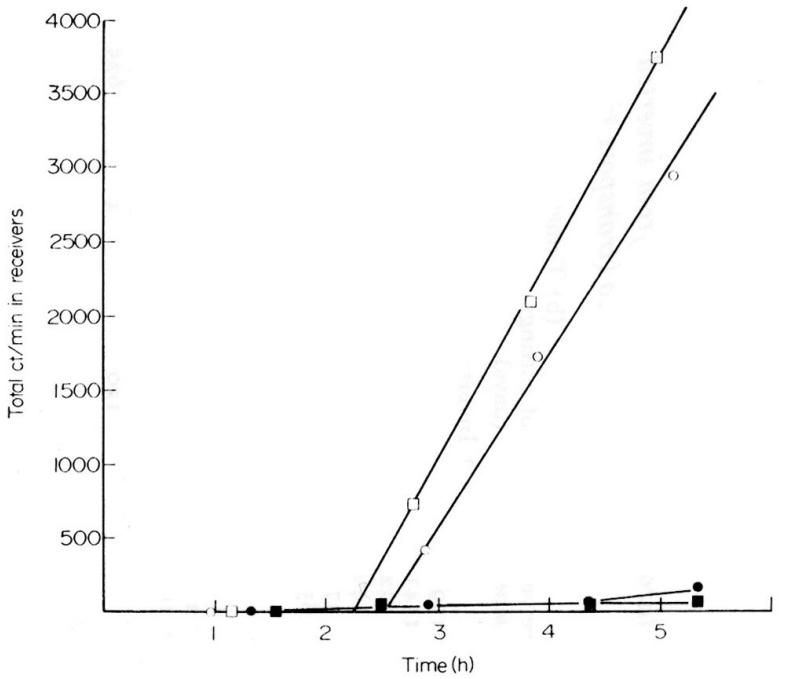

Fig. 1. Rates of auxin trrnsport by internode segments (1 cm long; three segments per measurement) from 85-day-old cuttings of tomato plants. Circles, inverted; squares, control; open symbols, basipetal; filled symbols, acropetal.

Similar experiments were carried out with inverted cuttings of tomato plants. The roots and shoots of these cuttings developed well (Plate 1, No. 1) and the shoots eventually flowered and bore fruit (Plate 1, No. 2). Even after 120 days development in an inverted position, internode segments from these plants transported auxin predominantly basipetally (representative results are shown in Table 2b). Some acropetal movement took place, but little or no more than in segments from control plants. The rates of acropetal and basipetal auxin movement in segments from an inverted and a control cutting (both 85 days old) are shown in Fig. 1.

Inverted cuttings of tomato plants.
No. 1. 51 days after inversion.
No. 2. 92 days after inversion.

A microscopical examination of cross-sections of the stems of inverted tomato cuttings revealed that considerable cambial activity had taken place; after 88 days the amount of secondary xylem was about double that present in the cuttings at the time they were inverted.

Inverted cuttings of tobacco plants did not develop as quickly or as well as inverted cuttings of *Tagetes* and tomato. Nevertheless roots and shoots were formed and some of the shoots grew to a length of about 20 cm before flowering. Auxin transport in internode segments of these plants was strictly basipetal even after 120 days growth in an inverted position (Table 2c).

Discussion

I was unable to obtain any convincing evidence for the acropetal transport of auxin in internode segments from dozens of inverted cuttings even after 4 months growth. Only in the apical parts of the internodes of *Tagetes* cuttings which had been treated with solutions of IAA was there any considerable acropetal movement of auxin, but these segments looked unhealthy and the pith within them had decayed. This acropetal movement could have taken place by diffusion along the surfaces of the cavity within the stem or simply reflect a decline of polarity in the tissues. It is well known that the polarity of tissues declines as they age, and auxin moves by diffusion equally well in both directions in dead tissues (Chang and Jacobs, 1972).

Went (1941) carried out three series of experiments on inverted cuttings, the first with cuttings which had been transported from Maryland to California which he described as 'not in the best condition'; auxin moved more or less equally well in acropetal and basipetal directions. In a second series of experiments on inverted *Tagetes* and tomato cuttings he found that 'the original polarity was still much in evidence in the inverted cuttings, and only a few indications of inverted auxin transport were found'. In the third series of experiments he used *Tagetes* plants which had been treated with solutions of IAA in order to induce rooting. Some acropetal movement of auxin was found in internode segments from these plants tested 21–66 days after inversion, but even so the amount moving acropetally was less than half the amount that moved basipetally. In these segments auxin probably moved acropetally by diffusion, possibly through decayed pith.

In secondarily thickening stems the majority of the polar auxin transport takes place in the newly formed cells of the secondary tissues (Sheldrake, 1973a). The basipetal polarity of auxin transport in the stems of inverted cuttings therefore indicates not only that the original polarity persisted in the cells originally present in the cuttings, but that the cells formed after inversion of the cuttings had the same polarity as their mother cells or neighbouring cells. This polarity was maintained in spite of the reversal of all the physiological gradients which depend on the relative positions of the roots and shoots. Thus the polarity of auxin transport in the stems of inverted cuttings provides a striking confirmation of the hypothesis that there is an autocatalytic maintenance of polarity associated with the differentiation of vascular tissues.

Auxin is produced in secondarily thickening stems as a consequence of vascular differentiation (Sheldrake and Northcote, 1968; Sheldrake, 1971; Sheldrake, 1973b). The patterns of growth and differentiation in the cambial region therefore maintain themselves in two related ways: vascular differentiation leads to the production of auxin which leads to further cambial activity and vascular differentiation (Sheldrake and Northcote, 1968), and the polar transport of auxin maintains the polarity of the newly formed cells.

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