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by

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Polar Transport of Radioactivity from C^{14}-Labelled-β-Indolyl acetic acid in Stem Segments of Pisum Alaska Vilmorin

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The results presented in this paper show that, in stem segments of Pisum, there is a predominantly basipetal movement of C^{14} from applied IAA labelled with C^{14}. The effect of gravity is quite evident and this shows that IAA, applied in receiver blocks, bears the action of gravity.

INTRODUCTION

The translocation of endogenous auxin in intact plant organs and the movement of exogenous β-indolylacetic acid (IAA) in organ cuttings have given rise to numerous publications (see for instance Jacobs, 1961; Pilet, 1961; McCready, 1966). All these studies have permitted the clarification of the polarity of auxin transport both *in vivo* and *in vitro* (Pilet, 1968b).

With the use of IAA labelled with C^{14} (IAA*), the analysis of the IAA movement, applied in accordance with the previous techniques of Went (1928) and Van der Weij (1932–1934) to plant segments in agar blocks (donor), has been greatly facilitated (Pilet, 1965).

The aim of this work is to analyse the auxin transport in stem sections as regards gravity effects.

The present work was undertaken in the Institute of Professor Pilet in which I had the opportunity of working for several months.

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MATERIAL AND METHOD

The method used was primarily the same as Went and Van der Weij, in which an agar block (donor) including IAA* is applied to one end of a section cut from a stem of *Pisum sativum* (Alaska Vilmorin). The movement of IAA through the section is estimated by measuring the C^{14} appearing in an agar block (receiver) and applied to the other end of the section, according to the technique described, for the *Lens* stem segments by Pilet (1965).

Seeds were first soaked for 24 hours with deionised water and kept in an incubator at 25°C., then washed with running tapwater for 6 minutes and then placed in Petri dishes on a cotton-wool and filter paper with deionised water to germinate, at a constant temperature of 25°C. Seedlings were used when five days old and selected as about 20 mm long. All experiments were carried out at 25°C in complete darkness. Only green light was used during manipulations. IAA-2-C^{14} (kept in an acetonitrile solution) was used with a specific radioactivity of 13,3 mc/mM.

0.4 ml solution of the labelled acetonitrile (IAA* 1,3 mg/25 ml in aceto nitrile), was evaporated under vacuum, then water (5 ml) was added, heated for four-or five minutes. Then 75 mg agar was added and mixed in a boiling water bath (% 1.5 agar). The solution was placed in a glass tube in which it was allowed to solidify. The agar cylinder formed was pushed out of the tube and divided into blocks with a special guillotine (diameter: 2.64 mm; length: 2.38 mm).

Technique for the preparation of the segment has already been described by Pilet (1965). Segments of 6 mm were cut with a special guillotine and placed on a support in horizontal position. The blocks supplying the sections, which are referred to as the donor blocks, with C^{14} from IAA* can be placed on the apical or on the basal cut surface and the blocks collecting C^{14} which went through the sections (receiver blocks) can be applied on the opposite cut surface. 20 segments on four supports constituted a treatment. The supports with segments were kept in a moist atmosphere (dark, 25 $\mp$ 0.5°C) during the experiments. Each of the twenty segments involved in one treatment were removed care-

fully at the end of the experiment, and split centrally into an upper and a lower half as accurately equal as possible. The upper and lower halves were cut separately into four pieces (8 sections) fig.1. Sets of 20 blocks or 20 sections were put on separately numbered small boards and put into a dessicator under the vacuum for 24 hours. Of each sample 1 ml. absolute alcohol solution was then spread evenly over the planchette and the sample re-dried in the dessicator for 24 hours before counting. Determination analyses were made at an end-window of a Geiger-Müller counter for radioactivity. All results are expressed in corrected disintegrations and per minute (DPM).

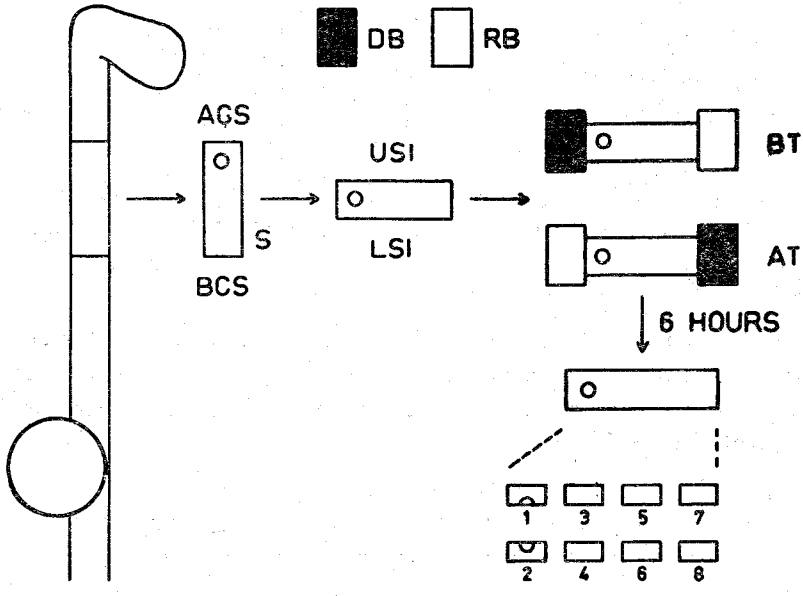

Figure 1. Preparation of sections Pisum Alaska Vilmorin.

S. segment of the stem.
ACS. Apical cut surface; BCS. Basal cut surface.
DB. Donor Block; RB. Receiver Block.
US. Upper side; LS. Lower side.
BT. Basipetal transport; AT. Acropetal transport.
1–8. Sections of the segments.

RESULTS

Polarity of the movement of C^{14}.

In the first series of studies two different experimental conditions were employed (for details see fig. 1) in order to investigate the effect of the orientation of the sections on the basipetal and the acropetal transport of C^{14}. Results are shown in Table 1 from which the following can be concluded: there is a typical polar movement of C^{14} from applied IAA. The basipetal translocation of C^{14} is predominant, which indicates that IAA moves essentially from the physiological apex of the stem to its base.

TABLE 1

Radioactivity	DPM/Block	
	Basipetal	Acropetal
In donors (D_0)	14360.6	14360.6
(D_6)	1303.9	3014.4
$\Delta D = D_0 - D_6$	13056.7	11346.2
(R_6)	156.3	16.2
R_6 % of ΔD	1.20	0.14

Polarity of the movement of C^{14}. Diffusion time 6 hours. Specific activity 13.3 mc/mM of the IAA-2-C^{14}. Each result the average of D_6 and R_6: 10 blocks; D_0 5 blocks.

Kinetic of the basipetal movement of C^{14}.

To analyse the intensity of the IAA* translocation - for the basipetal movement - a few experiments (see fig. 1) and several calculations were made (according to Pilet, 1968a). Results are presented in Table 2.

TABLE 2

Total Radioactivity	DPM/per block
In donors, (D_0)	13690.4
(D_6)	1502.9
Loss from donors, $D = D_0 - D_6$	12187.5
(R_6)	103.5
Calculated in segments $\Delta r = \Delta D - R_6$	12084.0
Measured in segments (r)	7696.4

Total calculated and measured radioactivity for the basipetal movement. Diffusion time: 6 hours. Radioactivity in DPM/per block. Each result the average of D_6, R_6: 160 blocks. D_0: 40 blocks.

Basipetal movement of C^{14} in relation to gravity.

As can be seen in fig. 1, each segment after a horizontal position of 6 hours, was first cut in two longitudinal sections: the upper section (US) and the lower section (LS). Then, each section was cut into 4 pieces: 1,3,5 and 7 for the upper part, 2,4,6 and 8 for the lower part. The absolute distribution of C^{14} will be first presented (Table 3), then relative values will be calculated (Tables 4, 5 and 6). From these results, it can be concluded that:

TABLE 3

US/LS	1/2	3/4	5/6	7/8	1+3+5+7/2+4+6+8
US	2053.7	887.0	432.3	415.8	3788.8
LS	2030.0	935.3	471.0	471.3	3907.6
US+LS	4083.7	1822.3	903.3	887.1	7696.4

Basipetal movement of C^{14} in relation with gravity (Absolute values: DPM/section) (ses fig. 1).

Each result average of 160 sections

US : upper sections

LS : lower sections.

TABLE 4

US/LS	1/2	3/4	5/6	7/8
USa	50.29	48.67	47.86	46.87
LSb	49.71	51.33	52.14	53.13

a) $\% \frac{US}{US+LS} 100$ b) $\% \frac{LS}{US+LS} 100$

Basipetal movement of C^{14} in relation to gravity (relative values: %) (see table 3).

TABLE 5

US/LS	1/2	3/4	5/6	7/8
USa	54.20	23.41	11.41	10.97
LSb	51.95	23.94	12.05	12.06

a) $\% \frac{US}{1+3+5+7} 100$ b) $\% \frac{LS}{2+4+6+8} 100$

Basipetal movement of C^{14} in relation to gravity (Relative values: %) (see table 3).

TABLE 6

US/LS	1/2	3/4	5/6	7/8
USa	26.68	11.52	5.61	5.40
LSb	26.37	12.15	6.12	6.12

a) $\% \frac{US}{1+2+3+4+5+6+7+8} 100$ b) $\% \frac{LS}{1+2+3+4+5+6+7+8} 100$

Basipetal movement of C^{14} in relation with gravity (Relative values: %) (see table 3).

1) radioactivity is greater (except for the two first sections) for the lower sections than for the upper one.

2) There is a constant decrease of IAA* from the cut section on which IAA was applied (donor) to those placed immediately in contact with the receivers.

CONCLUSION

The results presented in this paper show that, in stem segments of *Pisum*, there is a predominantly basipetal movement of C^{14} from applied IAA labelled with C^{14}. These assays also show that if there is a typical translocation of C^{14} from the physiological apex of the stem segment to its base, an acropetal transport of slight intensity cannot be excluded. Pilet recently (1968a), using stem stegments of *Lens*, discussed the relative importance and the physiological significance of two types of IAA movement. It does appear that the *Pisum* segments possess, as regards auxin polarity, the same characteristics as those of *Lens*.

The effect of gravity, on the other hand, is quite evident, and this shows that IAA, applied to the apical cut section, bears the action of gravity - as has already been demonstrated in the first studies dedicated to those questions (see Pilet, 1961, Leopold, 1961, McCready, 1966).

The Author wishes to express her gratefulness to Professor P. E. Pilet, director of the Biology and Plant Physiology Institute of the University of Lausanne, for his wide advice and his help during the actuation of this study.

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ÖZET

Bu araştırmanın maksadı, yerçekimi etkisi altında sürgün parçalarında auxin iletimini incelemektir. Neticeler Pisum sürgünlerinde C^{14} - işaretli -β- Indolyl acetic acid'in predominant olarak basipetal hareket ettiğini ve apical kesitlere tatbik edilen IAA iletiminde yerçekimi etkisi olduğunu göstermiştir.

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