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NON-POLAR TRANSLOCATION OF ABSCISIC ACID IN PETIOLE SEGMENTS OF COLEUS

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SUMMARY

Transport and distribution of abscisic acid (ABA) and of naphthaleneacetic acid (NAA) within segments of *Coleus* petioles was studied. Transport of ABA is slow compared with that of NAA and is non-polar. Net loss of ABA from donor blocks is small and of the same magnitude as loss of mannitol from donors. Distribution inside tissue segments is identical for ABA and mannitol; the distribution of auxin is characteristic.

1. INTRODUCTION

Polarity in plant morphogenesis is a well-known phenomenon. A number of morphogenetic processes like apical dominance and abscission can be explained on the basis of polar movement of plant hormones. Studies on hormone translocation are important for these morphogenetic processes and may also elucidate the primary step in hormone action. This is particularly true of auxins, as it has been suggested that processes involved in auxin transport are closely linked to or even identical with the primary action of auxin (HERTEL et al. 1969).

The transport system of synthetic auxins and natural auxin in stem and petiole tissue of *Coleus* has a number of characteristics (VEEN 1966, 1967, 1969, 1972, VEEN & JACOBS 1969a). No polarity in transport of radioactively labelled kinetin was observed (VEEN & JACOBS 1969b). Other workers (e.g., BLACK & OSBORNE 1965) found transport of cytokinins to be polar. The diversity of opinions on transport characteristics of plant hormones can be extended over other groups of hormones, as reviewed by ZIEGLER (1973).

Polar basipetal translocation of abscisic acid (ABA) in *Coleus* has been demonstrated by DÖRFFLING & BÖTTGER (1968). A velocity of 24–36 mm/h was found. MILBORROW (1968) reported basipetal transport 3 times as great as acropetal transport. INGERSOLL & SMITH (1971), working with radioactively labelled ABA, confirmed Dörffling and Böttger's value for the velocity. However no polarity was found in their cotton petiole segments. They suppose that indoleacetic acid (IAA) and ABA transport mechanisms are essentially the same, and even perhaps the transport mechanism for all hormones.

More recently, transport of ABA has been studied in segments of pea seed-

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lings by DÖRFFLING et al. (1973), who observed no polarity in the translocation, contrary to their earlier results with *Coleus*. The velocity was rather low, 2.4 mm/h for basipetal movement and 3 mm/h for acropetal movement.

These conflicting results on ABA translocation encouraged us to re-investigate transport properties of ABA in *Coleus* petiole segments.

2. MATERIALS AND METHODS

2.1. Plant material

All material originated from a single clone of *Coleus scutellarioides* Bentham propagated vegetatively over the years and formerly used by GORTER (1964) and VEEN (1966, 1972). The plants used in the experiments were grown in a controlled growth room under the following conditions: daylength 17 h; irradiance 25 W m^{-2}; air temperature and relative humidity 25 °C and ca. 60 % respectively, during day and night. Axillary buds were removed at regular intervals to promote uniform development. The petiole segments used for transport experiments were taken from plants when they had five internodes. The youngest leaf pair Leaf-Pair 1 was taken as that with petioles at least 5 mm long.

2.2. Chemicals

a. Non-radioactive synthetic abscisic acid, mixed isomers, was purchased from Sigma Chemicals Comp. (St.Louis).

b. Abscisic acid- (1–^{14}C) was purchased from Mallinckrodt Chem. (St.Louis) and had a specific activity of 1.64 mCi/mmol. Thin-layer chromatography on silica gel plates with GF 254, with distilled water as a solvent system and gas chromatography (*fig. 1D*), showed that the original material was mainly the cis-isomer, which strongly inhibited the germination of flax seeds. The identity of this radioactive compound was further confirmed by conversion into the trans-isomer: the cis/trans ratio in the original stock was 91:9 checked by radioactive analysis; after illumination under weak daylight for about 2 months, this ratio was 67:33.

c. α-Naphthaleneacetic acid-(1–^{14}C) (NAA), sp.activity 50.6 mCi/mmol; mannitol-(1–^{14}C) sp.activity 20.5 mCi/mmol; 3-0-methyl-(D-glucose-^{14}C(U)) sp.activity 19.0 mCi/mmol (Me-Glc). These compounds were all purchased from Radiochemical Centre, Amersham, England.

All compounds (except ABA) were dissolved in demineralized water by agitating the solution in an ultrasonic cleaning bath for 10–15 min. The pH of the agar blocks was about 5.2. ABA was prepared as the sodium salt; it was dissolved in a few droplets 0.1 M-NaOH and subsequently neutralized by 0.1 M-HCl.

2.3. Transport studies

Except for a few minor modifications procedure was as previously (VEEN 1972). Petiole segments were 1 cm long. Radioactive compounds were applied in donor blocks with a volume of 26 µl. The movement of the radioactive isotope was

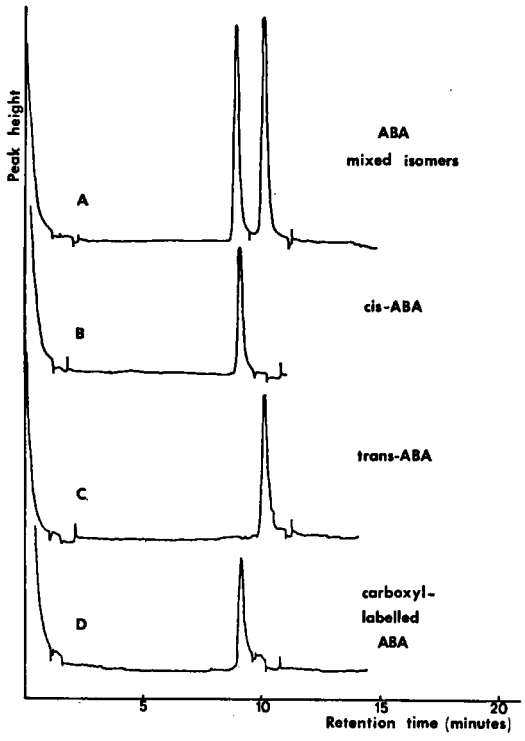

Fig. 1. GLC of A) BSTFA-silylated synthetic abscisic acid from Sigma (mixed isomers); B) and C) the isolated cis and trans isomers of the same compound; and D) radioactive labelled abscisic acid from Mallinckrodt Chem. Chromatographic conditions were: 4% O.V.-1 as a stationary phase on gas chrom. Q in a 1.75 mm internal diam. X 1.5 m Pyrex column; N_2 flow rate 30 ml/min (outlet); flame ionization detection. Temperature program: 3 minutes isothermal at 150°C, then increased by 10°C/min for 6 minutes, then isothermal for 5 min.

estimated by assaying the radioactivity accumulated in the receiver blocks, which had the same volume as the donors. Donors and receivers were assayed at the end of an experiment, so that transport could be expressed as a percentage of the amount lost from donors in the same period. At the end of a period, the segments were cut transversely into four parts, each 2.5 mm long. These parts were frozen in dry-ice and extracted in 50% aqueous ethanol. The radioactivity was counted in a liquid scintillation spectrometer.

All experiments were repeated several times, with 5–10 segments for each treatment. The significance of the difference between the treatments was checked with a t test. Regression analysis was by standard procedure (BAILEY 1959).

3. RESULTS

3.1. A comparison between auxin and ABA transport

To draw a parallel between different groups of plant hormones, test series for ABA were always accompanied by series for auxins, for which many transport properties have been established. The radioactively labelled compounds were applied in concentrations of 13 μM for NAA and 43 μM for ABA. The total radioactivity (expressed as disintegrations per minute, min^{-1}) in donor blocks at time of application is indicated by D_0; D_t and R_t represent the radioactivity

Table 1. Time course of basipetal and acropetal movement and apical and basal loss of ^{14}C added as NAA-^{14}C and as ABA-^{14}C to petiole segments of Leaf Pair 2. Initial radioactivity in donor blocks (D_0) was 37646 min^{-1} for NAA and 4081 min^{-1} for ABA. Initial concentration in donor blocks was for NAA 13 μM and ABA 43 μM. The data (corrected for background) are presented as means of 5 samples in disintegrations per minute.

	Transport time in h	D_t	ΔD	$100\,\Delta D/D_0$	R_t	$100\,R_t/\Delta D$
NAA						
basipetal	4	30360	7286	19.4	1009	13.8
transport	6	25647	11999	31.8	1917	15.9
	8	24543	13103	34.8	2450	18.6
acropetal	4	36127	1519	4.0	1*	0.0
transport	6	35366	2280	6.0	3*	0.0
	8	34322	3324	8.8	1*	0.0
ABA						
basipetal	4	3982	99	2.4	0	0.0
transport	6	3887	194	4.8	1*	0.0
	8	3854	227	5.5	1*	0.0
acropetal	4	3730	351	8.6	0	0.0
transport	6	3667	414	10.1	1*	0.0
	8	3556	525	12.8	0	0.0

* not significant

(in min^{-1}) in donors and receivers after transport period t. The difference between D_0 and D_t ($= \Delta D$) divided by D_0 is called relative net loss from donor ($100\,\Delta D/D_0$). The radioactivity in receivers is expressed as a percentage of ΔD: $100\,R_t/\Delta D$.

The data for a typical transport experiment are given in *table 1*. These data show that only NAA moves basipetally. In agreement with earlier observations (VEEN 1972), NAA shows an absolute polarity. The net loss from the donor is significantly less acropetally than basipetally, so reflecting the polarity of the transport system. To estimate the velocity of transport of NAA, a linear regression is fitted to the data for basipetal transport. The equation is:

$$y = -369.5 + 360.2\,x$$

in which y represents radioactivity in the receiver in min^{-1} and x time in hours. The calculated velocity of the basipetal transport from the extrapolated regression was 9.7 mm/h. The data in *table 1* indicates that in experiments with ABA, relative net loss from donors in acropetal transport series is higher than in basipetally. This difference was not observed in replicates and can be explained in this particular case from morphology: the basal end of the segment had a greater cross-sectional area than the apical end.

To study the gradient inside the segment, the tissue was divided into four parts and extracted. The data from an experiment in which the distribution of ABA and NAA was studied in both directions for 6 h are shown in *fig. 2C* and *2D*. In basipetal direction, the NAA gradient shows the typical tendency to accumulate in the last 2.5 mm of tissue. Acropetal movement of NAA shows a very steep gradient, probably by recycling of the radioactivity (GOLDSMITH 1969).

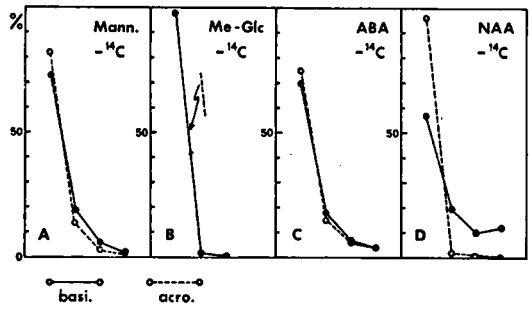

Fig. 2. A) Distribution of ^{14}C from Mannitol-1-^{14}C at a concentration of 11 μM (Mann.); B) 3–0 methyl-(D-glucose-^{14}C(U)), conc. 10 μM, (Me-Glc); C) abscisic acid-1-^{14}C, conc. 43 μM (ABA) and D) α-naphthaleneacetic acid-1-^{14}C, conc. 13 μM (NAA), along petiole segments 1 cm long. Both basipetal transport and acropetal transport have been studied for 6 hours. Radioactivity in the 4 parts is expressed as a proportion of the total in the segment.

The gradients of ABA are almost symmetrical and demonstrate the non-polar nature of the movement. The polarity was further expressed as the quotient of the amount transported basipetally to the amount transported acropetally for any given distance of transport (DE LA FUENTE & LEOPOLD 1966). In agreement with these authors the polarity quotient (Q) increases in an exponential way with increasing distances from the donor block (*fig. 3*). The data (x = distance from source; y = log Q) fits linear regressions with the equation:

$$\log y = -1.0703 + 1.2454x$$

for NAA transport after 6 h and

$$\log y = -0.6870 + 0.8658x$$

for NAA transport after 8 h. LEOPOLD & HALL (1966) used these relationships to demonstrate that a weak polarity in auxin transport in each cell is sufficient to achieve absolute polarity in a tissue. Our own data show that there is a tendency for polarity to decrease with time. The polarity quotient for ABA translocation was not significantly different from 1, so that polar movement was absent.

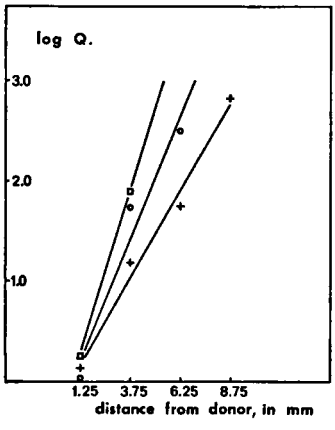

Fig. 3. Increase in polarity quotient (Q) with increasing distances from donor block. NAA transport was studied for 4 h(□—□), 6 h (0—0) and 8 h (+—+).

3.2. Studies on uptake processes

The absence of radioactivity from ABA-^{14}C in receiver blocks, even after a transport period of 8 h, can be explained in at least two ways:

a) ABA is not taken up in the cytoplasm and therefore movement is restricted to the apoplast

b) ABA is taken up into the cytoplasm either actively or passively, and is there fixed in some way and is not readily available for transport.

To discriminate between these alternatives, the following experiments were made. In these experiments, ABA, NAA, mannitol and methylglucose were used. It may be assumed that the mannitol molecule cannot pass the outer membrane; its movement is therefore limited to the apoplast. Methylglucose was used by REINHOLD & ESHHAR (1968) to study sugar uptake; it competes with glucose for the same carrier sites but is not metabolized, so that uptake processes can readily be studied. The data in *table 2* indicate that the smallest relative net loss from donor blocks was in the mannitol series (5–6%). This value can be attributed to filling of the free space in the tissue. ABA shows an uptake of 7–9% equal to that of NAA transported acropetally. Basipetal transport of NAA shows a relative net loss of about 18%, while methylglucose, which passes the outer membrane quickly, shows a value of nearly 50%. Only with basipetal transport of NAA did significant amounts of radioactivity reach the receivers. From these data, it looks as though the low loss of radioactivity from ABA-donor blocks (ΔD) can be explained principally as filling of the free space in the tissue.

The distribution within the tissue is shown in *fig. 2*. Mannitol, methylglucose and ABA show symmetrical distributions, evidence for a non-polar nature of their movement. Methylglucose shows a steep gradient similar to that of NAA transported acropetally. This is a further indication that methylglucose is fixed

Table 2. Basipetal and acropetal movement and apical and basal loss of ^{14}C added as Mann. = Mannitol-1-^{14}C (11 μM), D_0 = 10955 dpm; Me-Glc: 3-0 methyl-(D-glucose-^{14}C (U)), (10 μM), D_0 = 9537 dpm; ABA: Abscisic acid-1-^{14}C (43 μM) D_0 = 4159 dpm and NAA: -naphthaleneacetic acid-1-^{14}C (13 μM), D_0 = 36956 dpm. Transport period was 6 h; basipetal and acropetal movement in 1 cm segments of Leaf Pair 4 was studied. Net loss from donor blocks (difference between D_0 and D_t) is expressed as a percentage of the initial amount. Transport (radioactivity in receiver blocks) is expressed as a percentage of net loss from donor blocks.

	net loss	transported
Mann. basipetal transport	5.3	0
Mann. acropetal transport	6.4	0.1*
Me-Glc basipetal transport	40.7	0
Me-Glc acropetal transport	44.4	0
ABA basipetal transport	7.4	0
ABA acropetal transport	8.7	0
NAA basipetal transport	17.9	13.2
NAA acropetal transport	8.3	0.2*

* not significant

very tightly within the symplast; 98 % of the total activity is present in the first 2.5 mm. The same high proportion extracted from the tissue adjacent to the donor block is found with acropetal movement of auxin, suggesting a recycling and subsequent immobilization of NAA, moving acropetally.

The gradient of ABA resembles that of mannitol, which suggests a passive uptake into the free space. There is, however, not yet published evidence from our long-term experiments on the metabolism of ABA that the hormone is taken up by the cell. It is likely within the short period of time in the transport experiments that the uptake into the symplast is of lesser importance than the filling of the free space.

4. DISCUSSION

Leaf abscission depends on a delicate balance between various plant hormones, in particularly auxin and abscisic acid. During leaf senescence, there is an increase of ABA relative to IAA in the leaf lamina and in the petiole distal to the abscission zone (DÖRFFLING & BÖTTGER 1968). This change in balance could be the cause of increased ethylene production and thereby the induction of hydrolytic enzymes in the abscission zone. The cause of such a shift in the hormone balance may be the decreased basipetal transport of auxin (VEEN & JACOBS 1969a).

However, transport capacity for ABA has also been shown to be age-dependent (INGERSOLL & SMITH 1971). In none of the experiments presented in this paper did significant activity reach the receiver blocks, although petioles of various ages were used. It seems therefore likely that auxin and not ABA transport is the controlling factor in the auxin/ABA balance.

Our results are strikingly different from those of INGERSOLL & SMITH (1971) and of DÖRFFLING & BÖTTGER (1968). The latter authors checked the presence of ABA in receiver blocks by a bioassay (*Avena* cylinder test). It is quite possible that other substances than the ABA applied, diffusing into receiver blocks, obscured the results. The discrepancy between the velocity of 22 mm/h found by Ingersoll & Smith and our data cannot be explained on such a basis. As they worked with (R, S)-abscisic acid, it could be that only the trans-isomer was transported. This seems unlikely as the cis-isomer is the physiologically active one. In some of our experiments, transport of the isolated trans-isomer was compared with that of the cis-isomer. No difference between the two isomers was observed. The discrepancy in the data can possibly be explained by the use of different plant material, although we did some experiments with a nondescript variety of cotton, in which no transport (radioactivity recovered in receivers) could be observed either.

From the data on net loss from donor blocks, it is presumed that ABA is first filling the free space. Uptake into the symplast can be a rate-limiting step showing a saturation level below the concentration in the experiments. The data therefore do not exclude the possibility of ABA movement in the symplast. The amounts passing the outer membrane within the short period may be too small

for significant movement to be observed. It should be further considered that radioactive material may be fixed, for instance inside the vacuole, and limits the amounts transported.

RUBERY & SHELDRAKE (1974) showed that the uptake of undissociated auxin molecules can be by passive diffusion (without a carrier). A carrier uptake applied for auxin anions. The high relative net loss of auxin from donor blocks in basipetal transport experiments can be explained by Rubery & Sheldrake's model, as the pH of our agar blocks is about 5.2, whereas the pK of NAA is 4.2. The NAA molecules will therefore to some degree be in the undissociated form and will diffuse in the cytoplasm. The pH of the agar block is in no way, however, an indication of the pH just outside the plasmalemna. In agreement with the suggestions of Rubery & Sheldrake, the lesser uptake of ABA can be explained by the way of preparing the donors. Due to the higher pH, ABA will be more dissociated. But a future paper will show that when auxins and ABA with respective labels of ^{3}H and ^{14}C are simultaneously applied in one donor, the absence of ABA transport and a polar movement of auxin could be confirmed. It therefore seems unlikely that differences in pH between donors can explain the differences in movement.

HERTEL et al. (1969) and VEEN (1972) have shown that the polar transport system is specific for physiologically active auxins and auxin analogues. MORRIS & THOMAS (1974) presented results suggesting that the long-distance basipetal transport system from the apical bud of intact plants is also specific for auxins. This is further evidence that this long-distance transport has much in common with that for auxins in isolated tissue segments.

DÖRFFLING et al. (1973) showed that "radioactive labelled ABA applied to the apical bud of intact pea seedlings moves only a small distance from the site of application, whereas indoleacetic acid applied in the same manner is translocated from the apex to the root, where it accumulates."

The driving force in hormone transport will not be discussed here. In a future paper an attempt is being made to simulate diffusion of auxin and abscisic acid in petiole segments of *Coleus* and see how far it could be the driving force.

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