Pattern of growth and ^{14}C -assimilates distributions in relation to photosynthesis in radish plants treated with growth substances

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Abstract

In a series of radish plants, with very thin hypocotyl and with a normal storage organ, the rates of photosynthesis, photorespiration and dark respiration did not differ. Therefore, the conclusion may be advanced, that translocation to the swollen hypocotyl is not determined by the photosynthetic productivity, but rather by the storage capacity. To check it this is connected with an unbalanced hormonal content, plants were treated with lanoline paste, with IAA, GA_3 , zeatin and all three in mixture or with injections of GA_3 -water solution into the swollen hypocotyl. In young radish plants, with high rate of growth of aerial parts, treatment with the above mentioned substances stimulated $^{14}\text{CO}_2$ -assimilation and increased retention of assimilates in ^{14}C -donors, probably owing to retardation of their senescence. It increased the competition for photosynthates between shoot and storage organ.

In older plants, in the stage of accumulation of nutrients in the swollen hypocotyl, IAA + GA_3 + zeatin did not affect $^{14}\text{CO}_2$ -assimilation, but in plants treated with growth regulators separately, assimilation decreased; IAA and GA_3 stimulated transport and accumulation of labelled substances in the swollen hypocotyl.

On the basis of experimental data the conclusion may be advanced that responsiveness of the particular organs and processes to growth regulators depends on the stage of plant development. Phytohormone did not change quantitatively the pattern of ^{14}C -assimilates distribution. They stimulated processes with preference for particular stages of development.

INTRODUCTION

On the basis of results presented in the previous paper (Starck, Ubyśz, 1976) the conclusion is advanced, that the rate of photosynthesis in radish plants does not seem to be under the control of sink activity

and that accumulation of nutrients in the storage organ is not limited by the rate of photosynthesis, but rather by the sink capacity. Therefore in the presented experiments sink capacity limitation was examined with the assumption, that low accumulation of nutrients in storage organs of some plants may be connected with an unbalanced hormonal content.

MATERIAL AND METHODS

Radish plants cv. Tetra Łowicka were grown in hydroponic culture, as in previous series of experiments (Starck, 1973; Starck and Ubyśz, 1976).

Estimation of CO_2 -assimilation was done on intact plants in infrared gas analysers — IRGA, in a closed system, under artificial light conditions. Photorespiration was calculated as the difference between CO_2 -assimilation rate in a nitrogen atmosphere with 1% of oxygen and under a natural atmosphere; dark respiration was measured under conditions of natural atmosphere.

Experiments involving the transport of ^{14}C -labelled assimilates were carried out in a plexiglass chamber, under natural light condition, during 20-min exposure. Distribution of ^{14}C -compounds was measured after 2- (expts. No. 1 and 3) or 4-hr translocation and also after 4 days (expt. No 2) and 96 hrs (expt. No. 1). Concentration of CO_2 during exposure ranged between 0.06 — 0.11% v/v with specific radioactivity 1.3 — 2.5 $\mu\text{C}/\text{mg}$ CO_2 . Sugars, soluble in 80% ethanol, were determined by phenol methods.

Radioactivity of plants labelled with ^{14}C (stored at -15°C till time of analysis) was estimated in 80% ethanol homogenates by the use of G-M gas-flow counter.

The methods of growth substances treatment are described separately in the particular experiments.

Most experiments included 3 or 4 replications (one plant per each). The differences between treatments were statistically analysed by Students *t* test.

RESULTS

Physiological characteristic of plants with limited storage capacity (Exp. No. 1)

The supposition that in radish plants simple feedback control of photosynthesis by acceptors does not exist was advanced on the basis of experiments done with intact and defoliated plants. The experiments presented in this paper were performed on intact, younger (23-day-old),

or older (32-day-old) plants obtained from two groups of seeds, collected from plants with small, thin hypocotyls (series A), and with a normal storage organ (series B)*. Plants from both series of seeds inherited their storage capacity from their mother plants. The average total mass of one plant was slightly higher in series B of both younger and older plants, mainly owing to the bigger storage organ. Their leaves and roots were smaller than in series A, with thin hypocotyls (Table 1, averages from 7 plants). In younger plants of series A, the storage organ constituted only about 15 percent of the total fresh mass of the whole plant, as compared to 35% in series B. The differences were even wider in older plants (9 and 49 percent, respectively).

Table 1

Contribution of particular organs to the total mass of two plant series used in experiments No. 1
(in g of fresh mass)

Age of plants	Series of plants	Blades	petioles	Storage organ	Roots	Whole plants
23-days	A	2.7	1.0	0.8	0.7	5.2
	B	2.7	0.6	2.1	0.6	6.0
32-days	A	5.1	2.8	0.9	1.2	10.0
	B	3.3	1.9	5.7	0.8	11.7

In younger plants the rate of photosynthesis, photorespiration as well as protein and sugar contents in blades did not differ in both series (Fig. 1). Similar results were obtained in experiments with labelled

Fig. 1. Characteristic of blades of plants with storage organ of various size (expt. No. 1)

A — series with small storage organ, B — series with large storage organ, P_b — gross photosynthesis, P_R — photorespiration, D_R — dark respiration

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carbon, where the $^{14}\text{CO}_2$ -assimilation rate in series A and B did not differ significantly (data not presented).

Export of ^{14}C -assimilates from the labelled blades, estimated after two and 96-hrs of translocation was higher in plants with a bigger storage organ (Fig. 2). The proportion of ^{14}C -substances translocated into the swollen hypocotyl in particular plants was determined by its size, as reported before (Starck, 1973; Starck, Ubyysz, 1976). This relationships was more pronounced in younger plants (Fig. 3).

Fig. 2. Distribution of ^{14}C -assimilates in younger (23-days) and older (32-days) plants as percentage of total radioactivity of the whole plant

A — series with small storage organ, B — series with large storage organ, Bl — blades, p — petioles, s. h. — swollen hypocotyle, r — roots, co — cotyledons (expt. No. 1)

The results of the above presented experiments support the evidence obtained before, that radish is not a source-limited plants. It is suggested, that some other factors regulate accumulation of nutrients in the radish hypocotyl.

Fig. 3. Relationship between the size of storage organs and their share in ^{14}C -assimilates exported from ^{14}C -donors. Translocation period — 2 hrs (expt. No. 1)

Effect of GA_3 on the pattern of ^{14}C -assimilates distribution (expt. No. 2)

The experiment was performed on plants with thickening hypocotyls contributing to the total fresh weight in about 50%. On the basis of Ricardo and Sovia's (1974) paper, growth of storage roots or hypocotyls, as well as accumulation of nutrients in those organs is regulated, among other agents, by invertase activity. According to Kaufman et al. (1968), Mac Milian (1972) and many others, GA_3 in short-term experiments enhances invertase synthesis. Vlitos (1974) reported that gibberellins stimulate accumulation of sugars in the stem of sugar cane. Therefore, in the reported experiments, GA_3 was introduced into the swollen hypocotyl to increase the mobilising power of the storage organ. Gibberellins were injected into the storage organ (10 μg per plant) in a volume of 100 μl as 10 small drops, with an Agla syringe, 4 hrs after $^{14}\text{CO}_2$ -exposure. Control plants were injected in the same way with water.

Fig. 4. Influence of GA_3 on the distribution of ^{14}C -assimilates (expt. No. 2)
 A — storage organ; B — roots

The effect of GA_3 treatment on ^{14}C -distribution and sugar accumulation was estimated 4 days after exposure to $^{14}\text{CO}_2$ (Fig. 4). Some difficulties in the comparison between the GA_3 - and H_2O -treated series were connected with the fact, that plants treated with GA_3 release about 16 percent less $^{14}\text{CO}_2$ in respiration than the control ones, whereas the

storage organ of GA₃-treated plants accumulated about 40 per cent more radioactivity and more sugars than the respective control one. The differences in roots radioactivity were small (below limit of error). The ratio of specific radioactivity of the storage organ to that of root increased as follows:

plants treated with: water	GA ₃
1.62	2.40

This points to the effect of GA₃ on the accumulation of nutrients in the storage organ.

Effect of growth regulators on growth, ¹⁴CO₂-assimilation and ¹⁴C-assimilates distribution (expt. No. 3)

One of the reasons of the high variability of the storage organ size, reported by Starck and Ubyysz (1976), may be an unbalanced hormonal content, which may limit accumulation of nutrients in the storage organ. According to the experimental work of Torrey and Loomis (1963) and others (review by Skene, 1975), auxins, cytokinins and gibberellins are factors stimulating cambial activity in radish roots. In previous experiments (Starck, 1973), kinetin did not affect accumulation of ¹⁴C-substances in the swollen hypocotyl. According to Radin and Loomis (1971), zeatin is the main cytokinin in radish plants; therefore in the experiments described with plants of two ages, the effect of GA₃, IAA, zeatin and a mixture of all three was examined. Hormones were introduced as lanoline paste, on the surface of the slightly wounded, upper part of the storage organ (at two points), in concentration calculated per 1 g of lanoline: 10 mg GA₃, 2 mg zeatin, 10 mg IAA or all mixed in the same proportion. Three days after treatment the plant with hormones, they were exposed to ¹⁴CO₂ in a plexiglass chamber.

In younger plants GA₃ stimulated growth of cotyledons and in a much smaller degree — of blades; (data not presented). All growth regulators used stimulated the rate of ¹⁴CO₂-assimilation (calculated per 1 g of fresh matter of blades and cotyledons, Fig. 5), but the highest effect was observed in IAA-treated plants. Blades of plants treated with GA₃, zeatin and hormones mixed together had a higher content of protein. In the series of younger plants, proteins also increased in cotyledons treated with hormones, especially with zeatin. Sugar content in the blades of younger plants increased only in the series treated with mixed hormones but in all series — in cotyledons.

The results reported above, suggest, that growth regulators affect ¹⁴CO₂-assimilation by retarding senescence of cotyledons and probably

also — of the blades. In the series treated with IAA, GA₃ and mixed hormones, lower export and higher retention of ¹⁴C-assimilates in the blades and cotyledons was observed (Table 2). This may be also connected with prolongation of their growth and retardation of senescence.

Fig. 5. Effect of growth substances on ¹⁴CO₂-assimilation and sugar and protein contents. „Y” — younger plants (21-days old), „O” — older plants (27-days old)

Of all ¹⁴C-substances transported from ¹⁴C-donors, the highest proportion of ¹⁴C accumulated in the storage organ, on the other hand, plants treated with hormones (zeatin excepted) exported less ¹⁴C-assimilates to that organ as compared with control ones. Hormones introduced to the upper part of the swollen hypocotyl did not produce a mobilizing center for assimilates in the treated area. The ratio of

Table 2

Translocation of ^{14}C -assimilates into storage organ and roots in radish plants treated with growth regulators (Expt. No. 3)

Organs analysed	Parameters examined	Younger plants (22-days)					Older plants (27-days)				
		control	+ IAA	+ zeatin	+ GA ₃	IAA + zeatin + GA ₃	control	+ IAA	+ zeatin	+ GA ₃	IAA + zeatin + GA ₃
Storage organ	fresh mass (mg)	2130	1505	2161	1821	1319	6252	7022	7509	6891	9261
	contribution to dry matter (%) of total plant	42.5	39.8	44.5	31.5	30.1	62.3	66.9	55.5	59.5	60.8
	contribution to radioactivity (%) of total plant	37.3	26.1	37.4	27.6	25.0	42.9	49.3	37.2	45.7	44.0
	ratio of specific radioactivity upper part: lower part	1.51	1.48	1.17	1.25	0.96	1.21	0.94	0.80	0.63	0.76
Roots	fresh mass (mg)	737	736	657	930	790	925	846	1217	875	1078
	contribution to dry matter (%) of total plant	14.7	19.5	13.5	16.1	18.0	9.2	8.0	9.0	7.5	7.1
	contribution to radioactivity (%) of total plant	14.6	16.6	10.7	13.8	16.9	8.7	9.0	7.3	6.2	7.1
percentage of total export to storage organ and roots		52	43	48	41	42	52	58	44	52	51

specific radioactivity of the upper to that of the lower part of the storage organ was the same (in IAA-series) and even lower than in the control series in the other experimental series.

In older plants (after abscission of cotyledons, which were already yellow), the effect of growth regulators was quite opposite. In all cases, except the series treated with mixed growth regulators, the rate of assimilation of $^{14}\text{CO}_2$ decreased. Nevertheless the sugar content increased in all but the IAA-treated series (Fig. 5). This suggests some changes in the activity of enzymes. The experiments of Huff and Ross (1975) on radish plants indicated, that zeatin enhances α -amylase activity and also that of other hydrolytic enzymes. The same is well known also for GA_3 .

Distribution of assimilates in older plants was affected by growth substances in another way than in younger plants (Table 2). Zeatine decreased transport to both acceptors, especially to the storage organ; IAA and, in a smaller degree GA_3 , increased ^{14}C -transport to the swollen hypocotyl. In no case, similarly as in younger plants, did growth substances create mobilising centers at the site of their application — the upper part of the storage organ. It may suggest, that they affected more generally the metabolism of the whole plant.

DISCUSSION AND CONCLUSIONS

On the basis of the experimental data described above, the conclusion may be advanced, that the responsiveness of the particular organs and processes to growth regulators depends on the stage of plants development.

In young, radish plants, with high rate of growth of aerial parts, treatment with IAA, GA_3 , zeatin or all these hormones in mixture, stimulated $^{14}\text{CO}_2$ -assimilation probably owing to stimulation of growth and delay of senescence of blades and in a higher degree, of cotyledons. It increased competition for assimilates between shoots and typical acceptors — the root and the storage organ.

According to Stoddart (1972), in mature leaves, a low level of GA-s limits the activity of RuDP-carboxylase and some other enzymes. In old pea leaves, a reduced capacity for $^{14}\text{CO}_2$ -fixation was observed in plants with experimentally lowered cytokinins level; this effect was reversed by application of cytokinins to the shoot (Mc David et al; 1973). An increase of assimilation of CO_2 was observed even on isolated chloroplasts treated with IAA; the effect was higher in the case of younger plants (Thomas et al., 1974).

In older plants, after abscission of the cotyledons, in the stage of accumulation of nutrients in the swollen hypocotyl, growth regulators,

introduced to plants as a mixture of IAA + GA₃ + zeatin did not affect ¹⁴CO₂-assimilation, but when the plants were treated with them separately, photosynthesis even decreased. At this stage the IAA and GA₃ content in plant tissue may limit accumulation of nutrients in the storage organ. Therefore exogenously introduced IAA and GA₃ stimulated ¹⁴C-accumulation in swollen hypocotyls. Zeatine did not affect storage capacity, because, as Radin and Loomis (1971) reported, at the stage of plants development, a high content of cytokinins both in the roots and storage organ was observed.

In all the presented data, hormones exogenously introduced did not changed quantitatively the pattern of ¹⁴C-assimilates distribution, but only stimulated processes, with preference of particular stages of their development: in younger plant — growth of aerial part and rate of photosynthesis and in older ones they affected accumulation of nutrients in the storage organ. Translocation sugars to the swollen hypocotyl is not determined by the rate of photosynthesis, which is almost the same in plants accumulating a great or small portion of nutrients in the storage organ. Therefore in radish as a sink-limiting plant, the capacity of the main acceptor seems to be determined independently of the photosynthetic productivity.

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*Zależność pomiędzy wzrostem i dystrybucją asymilatów a fotosyntezą
u rzodkiewki traktowanej regulatorami wzrostu*

Streszczenie

Opisane doświadczenia stanowią kontynuację badań, dotyczących fizjologicznych przyczyn różnej pojemności akumulacyjnej organu spichlerzowego rzodkiewki. Wykazano, że u roślin nie tworzących prawidłowego zgrubienia hypokotylu, zarówno intensywność fotosyntezy jak i fotooddychanie oraz oddychanie w ciemności, mają zbliżoną intensywność w porównaniu z roślinami akumulującymi substancje pokarmowe w organie spichlerzowym. Potwierdza to poprzednio wysuniętą sugestię, że rzodkiewka jest rośliną, u której plon rolniczy limitowany jest nie poprzez produkcję fotosyntetyczną, lecz przez pojemność akumulacyjną organu zapasowego.

Regulatory wzrostu, takie jak IAA (10 mg/g lanoliny), GA_3 (10 mg/g lanoliny), zeatyna (2 mg/g lanoliny) i wszystkie trzy fitohormony wprowadzone do rośliny łącznie w postaci pasty lanolinowej na zgrubienie hypokotylu, u młodych roślin (w okresie intensywnego wzrostu części nadziemnej) stymulowały asymilację $^{14}CO_2$. Wynikało to najprawdopodobniej z hamowania procesu starzenia się liści i liści. Ponadto IAA, GA_3 oraz IAA+ GA_3 +zeatyna zwiększały retencję ^{14}C -asymilatów w ^{14}C -donorach a jednocześnie hamowały ich transport do zgrubienia.

Rośliny starsze, w fazie intensywnej akumulacji substancji pokarmowych, wykazywały krańcowo różną responsywność na fitohormony. Obserwowano obniżoną asymilację $^{14}CO_2$. W serii roślin traktowanych IAA i GA_3 wykazano nieznaczna stymulację transportu asymilatów do zgrubienia. Podobny efekt wykazano w przypadku wprowadzenia do organu spichlerzowego roztworu GA_3 (w postaci iniekcji) już po zaznakowaniu roślin ^{14}C . Po 4-dniowym transporcie ^{14}C -asymilatów, gibberelina bardzo znacznie stymulowała akumulację ^{14}C -substancji w zgrubieniu.

Powyższe dane nasywają wniosek dotyczący bardzo dużych różnic w responsywności rzodkiewki na zwiększoną zawartość substancji wzrostowych. Fitohormony nie zmieniały w zasadniczy sposób wzoru dystrybucji asymilatów, a jedynie stymulowały proces, preferowany w danym etapie rozwoju rośliny.