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Sci. Rep. Kanazawa Univ.
Vol. 8, No. 2, pp. 511–521
September 1963

The Influence of Light on the Downward Transport of the Radioactive Carbon-Labeled Photosynthetic Products in Young Barley Plants

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(Recieved 30 June 1963)

INTRODUCTION

There are aboundant evidences that the photosynthesis rate is reduced by the accumulation of photosynthetic products in leaves, on the ground that leaves are only the assimilating tissue, but are not the organ of storage of the products. Part of sugars formed in leaves during photosynthesis was transported at once from leaves to stems. And starch formed during photosynthesis was decomposed sugars in leaves, and then the sugars were transported to other organs. The rate of those translocation was governed by the concentration of mobile compounds in leaves or in the phloem tubes. On the other hand, TURNER (8,9) has concluded, in his studies on the movement of organic nitrogen and carbohydrate, that the translocation of assimilated compounds can not readily be explained by the mass flow theory, but that may be determined by the prevailing concentration gradients of the mobile compounds in the phloem tissue.

If the movement of assimilated compounds from leaves to the other parts were to be affected by the concentration of sucrose in leaves, the rate of translocation of photosynthetic products would be affected by changing the metabolism in leaves. There have been many evidences that most of labeled compounds were accumulated in leaves as sucrose during the short term photosynthesis with $^{14}CO_2$, and that sucrose was the main form in which carbohydrates were translocated. Therefore, it was investigated in the experiments described below, whether the rate of translocation of photosynthetic products that was labeled with exposing $^{14}CO_2$ to leaves was affected by light or not. The radioactive carbon labeled compounds in roots have been identified by the chromatographic technique and compared with those in leaves.

MATERIALS AND METHODS

The plant materials used in all experiments were the young barley plants (Fujisaka No. 5) at the third leaf stage which had been raised in water culture with Hoagland's solution (pH 5.8-6.0) in a greenhouse about three weeks, as described by TAMAI and NISHIDA (7).

The plants were enclosed in an assimilation chamber and supplied with $^{14}CO_2$ (about 127 μc) generated from 18 *mg* of $Ba^{14}CO_3$ in closed circuit (Fig. 1). The

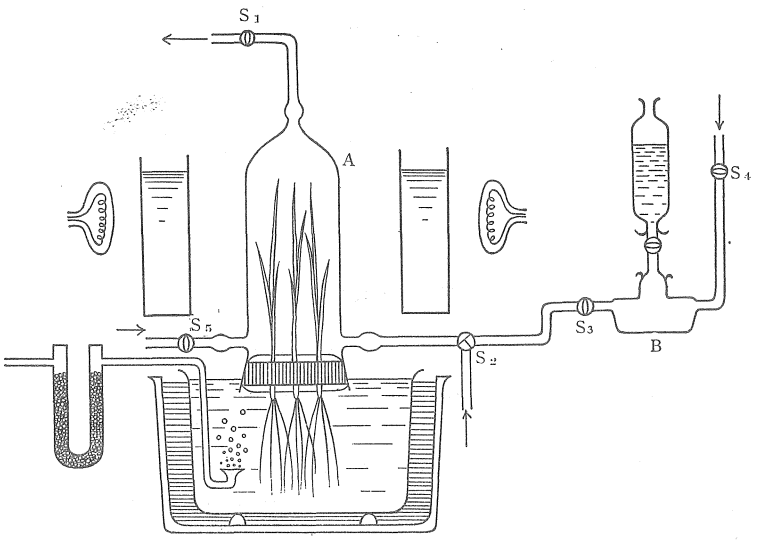

Fig. 1. Diagram of apparatus for photosynthesis with $^{14}CO_2$. The plants are set in an assimilation chamber (A) and sealed hermetically with acidic agar. Stopcock S_1 controls the reduced pressure by a vacuum pump in the chamber and in $^{14}CO_2$ gass generator (B). Stopcock S_2 and S_3 control the flow of $^{14}CO_2$ from the gass generator into the chamber. Stopcock S_4 controls the pressure in the chamber and the generator. After photosynthesis with $^{14}CO_2$ for thirty minutes Stopcocks S_1, S_2 and S_5 are opened, and $^{14}CO_2$ in the chamber is removed by a vacuum pump, and then $^{14}CO_2$-free air through the Stopcocks S_2 and S_5 is flushed in the chamber during the experimental periods. The nutrient solution is kept at 21°C and the aeration of the solution was continued for the whole experimental periods.

assimilation of $^{14}CO_2$ in the apparatus was carried out, except the time course experiment, for thirty minutes under the following conditions: CO_2 concentration in the chamber, 1 per cent; the room temperature, 21°*C*; light intensity, 30,000 lux of artificial illumination; the culture solution was kept at 21°*C* and the aeration of the solution was continued for the experimental periods. This treatment was called "*Pretreatment*". As soon as the pretreatment finished, $^{14}CO_2$ in the apparatus was removed, and then the plants were left out exposed to $^{14}CO_2$-free air for three or eight hours under the same light intensity or darkness and the other conditions were the same in the pretreatment.

At the end of the experimental periods the plants were cut to shoots and roots, and each sample was weighed. The sample was killed at once with hot 80 per cent

ethanol and homogenated by an Elvehjiem type homogenizer, and then this homogenate was kept overnight at, or near, 0°*C*. The radioactivity in a suitable aliquot portion of the homogenate was counted by a Geiger-Muller counter. Triplicate planchets were prepared, drying the sample together with a drop of 6 *N* acetic acid. Total assimilated radioactivity in shoots or roots was calculated as per 100 *mg* of fresh weight.

The other part of the homogenate was centrifuged, the residue was extracted with 20 per cent ethanol at 50°*C*. This procedure was repeated four times in order to extract completely. Total radioactivity in alcohol-soluble fraction was determined and calculated as total assimilated radioactivity was done. The other part of alcohol-soluble fraction was concentrated, to use for detection of labeled compounds in the extracts.

The labeled compounds in the alcohol-soluble fraction were determined with two dimensional chromatography. The chromatography was carried out on Whatman No. 4 paper (washed in 1 per cent oxalic acid) with phenol-water (500 : 125, w/v) as the first solvent and butanol-acetic acid-water (12:3:5, v/v) as the second. To detect radioactive compounds on the chromatograms, radioautograms were made by exposing the paper to Fuji PX X-ray film for a week, if the radioactivities were 20,000 counts per minute in the original spot. Radioactivity of compounds on the chromatogram was counted with a Geiger-Muller tube fitted with a "Mylar" window, and flushed continuously with Q gass (99.05% He and 0,95% isobutane). The labeled compounds on the paper, furthermore, were identified by co-chromatographing them to compare their R_f values with those of pure reference compounds.

The extraction and isolation of starch were carried out, using the method described by PORTER and MAY (5) and later modified in our laboratory. Firstly, acidic 95 per cent ethanol was added to the residue extracted with 80 per cent ethanol and warmed in a bath at 50-60°*C* for ten minutes; this was then centrifuged. The residue was washed three times with 95 per cent ethanol. The residue, removed of acidity, was extracted with 5 *ml* of water in a bath at 100°*C* for ten minutes. This extraction was repeated four times. The supernatant liquid contains the starch. The solution was made into slightly alkalinity with NaOH and concentrated to about 1 *ml* on a bath. Twenty milliliters of absolute ethanol were added to the concentrated solution and placed overnight at 20°*C*. The precipitated starch was separated from the alcohol, washed twice more with 95 per cent ethanol, and then resolved in 5 *ml* of water. Some of the precipitate that did not dissolve, was discarded by centrifugation. The starch was reprecipitated by adding ethanolic NaCl and IIK solution to the starch solution. The starch-I_2 complex was separated from the solution by centrifugation, decomposed with 0.25 *N* alcoholic NaOH, and centrifuged. The residue of starch was washed three times with 60 per cent ethanol. This purification of starch was done by ARONOFF's method (1). The starch

obtained was resolved in 5 *ml* of water, and this solution was used to count of the radioactivity in the starch.

RESULTS

The time course of the total activities of ^{14}C-labeled compounds which were accumulated in the roots while exposing $^{14}CO_2$ on leaves of barley plants at the third leaf stage, was investigated. The results were shown in Fig. 2. The ^{14}C

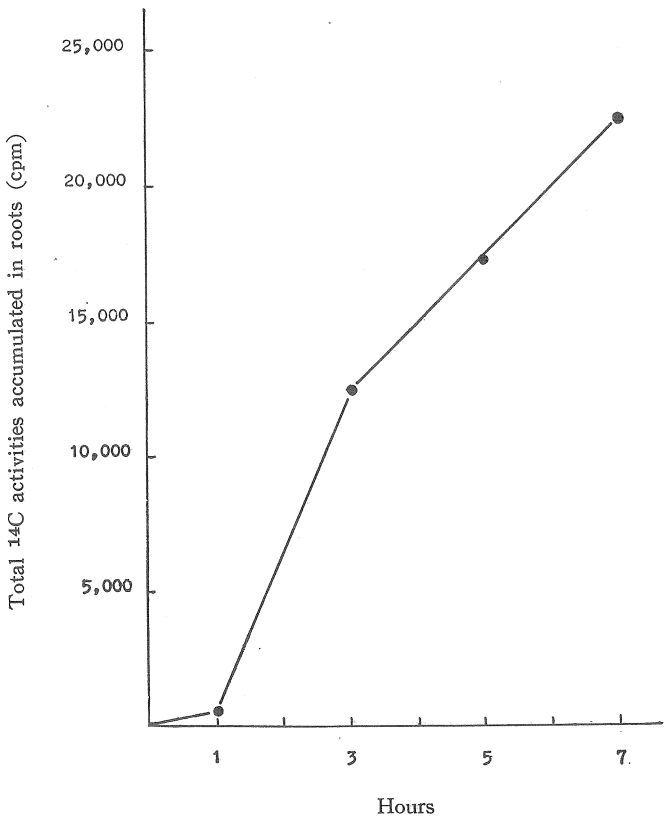

Fig. 2. The time course of the labeled photosynthates which were transported to roots during exposing $^{14}CO_2$ on the leaves of barley plants.

activity in the roots accumulated slightly within one hour, but increased rapidly from one till three hours, and then increased constantly. It seems that there is a time lag within one hour before the labeled compounds fixed by leaves were transported to roots.

After exposure of the leaves of barley plants to $^{14}CO_2$ for thirty minutes, namely pretreatment, was carried out, $^{14}CO_2$ in the apparatus was removed, and then the plants were kept in light or darkness, the leaves exposed to $^{14}CO_2$-free air. In the case of light, the ^{14}C radioactivities in the roots started to rise within one hour, reached a maximum in three to four hours, and then decreased with

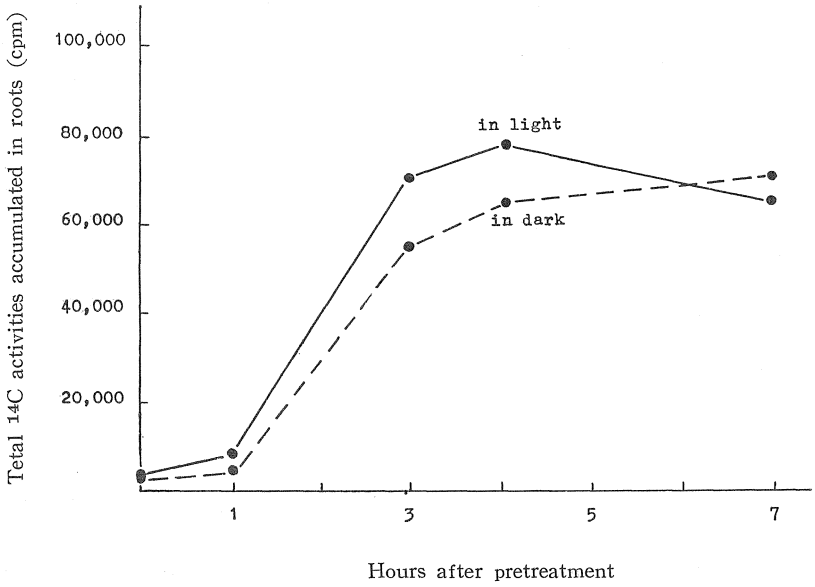

Fig. 3. Effects of light on the translocation of photosynthates which were fed on the leaves of barley plants for thirty minutes.

time (Fig. 3). On the other hand, in darkness, ^{14}C radioactivities remarkably accumulated in roots during the first three hours as in light, but, afterwards, those presented gradual increases with time. The total ^{14}C radioactivity accumulated in roots showed higher values in light than in darkness within four hours. It may show that most of mobile ^{14}C-labeled compounds fixed by leaves for thirty minutes were transported to roots during the first three hours, and that, especially in darkness, a suitable amount of mobile ^{14}C-labeled compounds was maintained in leaves still after three hours.

For the purpose of examining these light effects, the distribution of ^{14}C-labeled compounds in shoots and roots was investigated on the plants which had been kept in light or darkness for three or eight hours after the pretreatment. The results were shown in Table 1. The ratio between the amounts accumulated in roots and the total amounts in plants was 30.2 per cent in light for three hours, but, in light for eight hours, this ratio decreased to 23.8 per cent. In darkness, however, these ratios were 22.0 per cent at three hours and increased 29.0 per cent at eight hours. These transport ratios were nearly equal to the ratios that were determined by the radioactivity in the alcohol-soluble fraction. All photosynthates transported from shoots to roots during the pretreatment were included in the alcohol-soluble fraction. These results show that ^{14}C-labeled compounds in alcohol-soluble fraction may play a significant role in the transport of photosynthates. Though ^{14}C-activities in alcohol-soluble fraction in shoots were 95 per cent at the preteatment, they remarkably decreased to 68 per cent at three hours and furthermore decreased to 59 per cent at eight hours in light. In darkness, however, those

Table 1. The distribution of ^{14}C on shoots and roots of young barley plants kept in light and darkness after photosynthesis with $^{14}CO_2$.

	Pretreatment		Three hours after pretreatment			
	Light		Light		Dark	
	Shoots	Roots	Shoots	Roots	Shoots	Roots
Total accumulated ^{14}C activity, cpm *	427,400	1,100	294,500	127,200	302,500	85,100
Transport ratio, %		0.3		30.2		22.0
Total ^{14}C activity in alcohol-soluble fraction, cpm *	406,000	1,100	201,100	92,900	253,900	76,700
Transport ratio, %		0.3		31.6		23.2
Alcohol soluble fraction / Total accumulation, %	95.0	100.0	68.2	73.0	83.9	90.1
^{14}C activity in starch, cpm, *	4,200	0	5,500	1,700	4,600	1,100

* Radioactivites were calculated as per 100 mg of fresh weight.

^{14}C activities decreased to 83 per cent at three hours, but those decreases at eight hours were very slightly. the ^{14}C activities in alcohol-soluble fraction in roots also decreased greatly in light compared with those done in darkness. Both in shoots and roots in light, the radioactive carbon that was fixed in alcohol-soluble compounds during the pretreatment was incorporated remarkably into alcohol-insoluble compounds than in darkness. On the contray, in darkness, the ratio between alcohol-soluble and -insoluble fraction was constant in shoots, but this ratio in roots presented gradual decreases.

The incorporation of radioactive carbon into starch in shoots after the pretreatment increased at three hours in light and then decreased slightly at eight hours in light. The ratio of starch to the total ^{14}C activities in shoots hardly changed with time, although the ratio increased to 1.9 per cent at three hours and decreased to 1.8 per cent at eight hours in light, in comparison with 1.0 per cent at the pretreatment. In darkness for three hours, ^{14}C activities of starch in shoots were nearly equal to those at the pretreatment, but the ratio of starch to total ^{14}C activities in shoots increased to 1.5 per cent. In darkness for eight hours, however, both ^{14}C activities and the ratio in starch decreased greatly. On the other hand, in roots, the incorporation of radioactive carbon from alcohol-soluble into alcohol-insoluble fraction increased with time, especially remarkably in light after the pretreatment. Starch in roots was formed at three hours, but decreased at eight hours both in light and darkness, and the time course of radiocarbon activities was nearly equal to that of the ratio to total ^{14}C activity in roots. These results show that alcohol-soluble compounds may greatly incorporate into other alcohol-insoluble compounds besides starch both in shoots and roots under light condition, and that some metabolism in roots may be affected by the condition whether shoots are being exposed to light or not.

which had been

Eight hours after pretreatment			
Light		Dark	
Shoots	Roots	Shoots	Roots
285,600	89,300	225,000	92,100
	23.8		29.0
168,600	58,400	186,200	74,200
	25.7		29.7
59.0	65.4	82.8	80.6
5,200	900	1,700	800

And therefore, ^{14}C-labeled compounds in alcohol-soluble fraction were identified by two-dimensional decending paper chromatography. The results were shown in Table 2. In shoots at the pretreatment, the alcohol-soluble fraction consisted of about 83 per cent free sugar, about 10 per cent amino acids, about 4 per cent phosphate esters, and about 3 per cent organic acids. Sucrose was the only free sugar, most of amino acids consisted of glycine and alanine, most of the phospate esters were sugar phosphates, and malic and citric acid were the main compounds of organic

Table 2. The distribution of ^{14}C-labeled compounds in alcohol-soluble fraction in shoots and roots of young barley plants which had been kept in light and darkness after photosynthesis with $^{14}CO_2$.

	Pretreatment		Three hours after pretreatment				Eight hours after pretreatment			
	Light		Light		Dark		Light		Dark	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Total ^{14}C activity in alcohol-soluble fraction, cpm *x 10^3	406.0	1.1	201.1	92.9	253.9	76.7	168.6	58.4	186.2	74.2
Compounds ; % **										
sucrose	83.2	75.0	64.1	76.0	68.3	87.7	72.5	70.4	66.8	76.0
Total free sugars	83.2	75.0	64.1	76.0	68.3	87.7	72.5	70.4	66.8	76.0
sugar phosphates ***	3.3		1.7	2.4	5.1	4.4	1.8	1.3	4.8	6.7
Total organic phosphates	3.9		1.7	2.4	5.9	4.4	1.8	1.9	6.5	6.7
alanine	3.1	13.7	6.7	6.8	5.5	2.3	5.7	2.7	4.6	6.1
glycine	4.1	11.2	21.8	6.3	8.8	2.9	16.0	5.8	14.8	4.5
aspartic acid	2.1		0.5	1.4	3.0	1.1	1.0	1.5	2.1	4.4
glutamine			0.8	2.8	0.9	1.2	0.4	2.4	0.8	1.1
Total amino acids	9.9	24.9	29.8	18.2	23.4	7.9	23.1	15.7	25.6	16.1
malic acid	1.8		4.3	1.1	1.4	trace	1.6	4.0	1.0	0.7
citric acid	0.5		trace	trace	1.0	trace	0.6	trace	trace	trace
Total organic acids	3.0		4.3	1.1	2.4	trace	2.2	4.5	1.0	0.7
Unknown compound				2.3				7.7		

* Radioactivities were calculated as per 100 mg of fresh weight.

** Percent was calculated by summing up all the radioactive spots on the chromatogram.

*** Sugar phosphates include hexose-monophosphates and -diphosphates.

Total free sugars : Sum of the listed sugar only.

Total organic phosphates : Sum of the listed phosphates, including those not listed (phosphoenol-pyruvate, and phosphoglyceric acid).

Total amino acids : Sum of the listed amino acids, including those not listed (serine, glutamic acid, threonine, valine, leucine and unidentified ninhydrin-positive spots).
Total organic acids : Sum of the listed organic acids, including those not listed (glyceric acid, succinic acid, pyruvic acid, and fumaic acid).
trace : Indicates radioactive spot on film, but activity too low to count.

acids. On the other hand, only three compounds, namely sucrose, alanine and glycine, were found in roots at the pretreatment, and sucrose was 75 per cent. It may be said, therefore, that sucrose is the main compound of photosynthate which is transported from shoots to roots,and that alanine and glycine also are a transport form of photosynthates, for the intermediates in conversion of sucrose in roots are not found and, alanine and glycine are involved in large quantities as single compound, except sucrose, in shoots.

In shoots at three hours after the pretreatment, no essential difference of kinds of labeled compounds was observed between in light and in darkness. But the rate of these activities in light was significantly different from the rate in darkness. Especially, the rate of labeled sucrose was a small in light than in darkness, and amino acids, above all, alanine and glycine, had the high ratio in light. Glycine and alanine had about 95 per cent of total amino acids in light, but these compounds were about 60 per cent in darkness. Phosphate esters on light were less than those in darkness, and malic acid predominated in organic acids in light.

The distribution of labeled compounds in roots also showed much the same tendency as observed in shoots according to respective conditions of light. The rate of amino acids to alcohol-soluble fraction in roots was about 10 per cent higher in light than that in darkness. This was found to be based on increases of alanine and glycine. The activities of alanine and glycine in roots at three hours in light rised up 41.8 and 47.1 times, respectively, compared with those activities in roots at the pretreatment. But the increases of the activity of alanine and glycine in roots at three hours in darkness were slightly 11.7 and 18.1 times, respectively. On the contrary, the activities of sucrose in roots were not so significant as the difference observed on alanine and glycine between in light and in darkness.

From these results, the considerable difference of transport ratio between in light and in darkness may be regarded as being controlled according to the transported amounts of amino acids, above all, alanine and glycine. Of course, it admits of no dout that sucrose is the main compound transported from shoots to roots in barley plants as sucrose is 76 to 88 per cent of total alcohol-soluble fraction, but the role of amino acids also can not be neglected on the transport of photosynthetic products.

In the same way, the increase of the transport ratio at eight hours in darkness may be regarded as based on increases of alanine and glycine.

DISCUSSION

The identification of the translocates of $^{14}CO_2$-labeled photosynthetic products

has been carried out by many investigators, and they have pointed out that sucrose is the predominant translocated sugar (10, see also literature review in ZIMMERMAN, 12). BIDDULPH and CORY (2), with $^{14}CO_2$ supplied to a leaf of the red kidney bean, found that sucrose was the principal metabolite exported by the leaf, that labeled glucose and fructose were also present in the petiole although the exact proportion of each was not determined. VERNON and ARONOFF (10) concluded from their studies on soybean that sucrose, glucose, and fructose were all translocatory sugars, although the rate of translocation of hexoses was less than that of sucrose. However, SWANSON and EL-SHISHINY (6), with the concord grape, suggested that sucrose is the only translocated sugar, and that labeled glucose and fructose in the stem are hydrolytic products of the translocatory sucrose in the actual translocational channels. In our present experiments, sucrose was the only sugar in roots of barley plants at the pretreatment. Hexoses were universally absent. These results agree with findings of sieve tube exudate analysis of 16 North American tree species by ZIMMERMAN (11), though he found some trees carried, besides sucrose, sugars of the raffinose family of oligosaccharides, namely raffinose, stachyose, and verbascose. Phosphate esters, of which sugar phosphates were predominant, were present in shoots, but absent in roots at the pretreatment. BIDDULPH and CORY (2), feeding $Na_2H^{32}PO_4$ and $^{14}CO_2$ to a leaflet of the red kidney bean, found that a number of phosphate esters, of which fructose, 1-6, diphosphate was predominant, were present as was inorganic phosphate. But the fructose, 1-6, diphospate was not significantly carbon-labeled within 60 minute migration period, indicating that its carbon chain was not of current photosynthetic origin. And consequently sugar phosphates in roots at three or eight hours after the pretreatment are not identified whether they are translocatory compounds out of shoots or not. Therefore, it is considered that sucrose was the specific sugar of translocatory photosynthates in young barley plants.

Amino acids and amides are known to be translocated mainly out of leaves. TURNER (9) found that accumulation above the ringed zone was confined to the soluble nitrogen fraction and that amino acids and possibly peptides were involved in translocation. MITTLER (3,4) found aspertic acid, glutamic acid, serine, threonine, alanine, valine, leucine, and/or isoleucine, phenylalanine, asparagine, glutamine, and possibly γ-amino-butyric acid in the stylet sap of aphids that had been feeding on willow stems. In our present experiments, although many amino acids were found in shoots, alanine and glycine were found only in roots at pretreatment, and were regarded as amino acids that translocated mainly out of leaves. The considerable quantity of glutamine and aspartic acid were found in roots except at the pretreatment, but the available data are not sufficient to warrant any conclusion on the translocatory forms.

Whether the shoots were being carried on photosynthesis or not, affected not only the distribution of labeled compounds in shoots, but that in roots. In par-

ticular, it is one of absorbing interest that an unknown compound was observed in roots in light. But this compound is not yet identified. The difference of distribution of labeled compounds in roots at each treatment may be regarded as based upon the influence of light on root metabolism and upon the rate of translocatory compounds secreted out of leaves. Of these translocatory compounds in roots of young barley plants, the radioactivities of sucrose have no significant difference between in light and darkness for three hours, but those of alanine and glycine in light for three hours increased about fourfold and threefold, respectively, in comparison with that done in darkness for three hours. All radioactivities of sucrose and amino acids in roots decreased in light for eight hours compared with that of three hours, but, in darkness for eight hours, sucrose decreased and amino acids increased. It was found that both the total ^{14}C activity in roots and the transport ratio have higher values in light than that in darkness at three hours and lower values in light than in darkness at eight hours. These results may be considered to indicate that the movement of photosynthate is affected on how the transport rate of amino acids out of leaves. Of course, it need scarcely be said that sucrose is the main translocatory compound and assume the control of the transport amounts of photosynthates.

SUMMARY

Effects of light were investigated on the transport of the radioactive carbon-labeled photosynthetic products to the roots of young barley plants at the third leaf stage.

After photosynthesis with $^{14}CO_2$ for thirty minutes the plants were kept in light or darkness with the leaves exposed to $^{14}CO_2$-free air. The labeled compounds fixed photosynthetically on the leaves for thirty minutes remarkably accumulated in the roots during the first three hours both in light and in darkness, but, afterwards, those gradually decreased with time in light and increased in darkness. Both the total ^{14}C activity in roots and the transport ratio from shoots to roots showed higher values in light than in darkness within four hours, but lower values in light than in darkness at eight hours. The transport ratio that was obtained by the total ^{14}C accumulated in plants was nearly equal to the ratio determined by the alcohol-soluble fraction. And all photosynthates transported to roots at the end of photosynthesis with $^{14}CO_2$ (pretreatment) were found in alcohol-soluble fraction.

The distribution of ^{14}C was determined among the various compounds of the alcohol-soluble fraction that were separated by paper chromatography. Sucrose was the main compound of photosynthates which were transported out of shoots, and alanine and glycine also were the transport form of photosynthates. The significant effect of light was observed on the translocation of amino acids. But the

transport of sucrose was hardly affected by light. The results were obtained that light might affect greatly the incorporation of alcohol-soluble compounds in roots and shoots into other alcohol-insoluble compounds besides starch (may be perhaps protein), in particular, some root metabolism might be affected by the condition whether shoots were being exposed to light or not.

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