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STUDIES ON THE CARBON METABOLISM IN THE HIGHER PLANTS

II. STRUCTURAL DISTRIBUTION OF THE CARBON-14 ABSORBED THROUGH THE LEAF IN THE RICE PLANT

By

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Hitherto many reseaches have been carried on to clarify the mechanism of the biosynthesis and accumulation of the starch in the rice plant. In these reseaches, studies were made on determining the contents of the chemical constituents of each organs at each stage of their development, that is, to clarify the effect of the environmental conditions on the biosynthesis and translocation of starch from the analytical standpoint. Judged from the results, especially those concerning the behavior and trends of carbohydrate in the plant tissues, the role of each organ in various growth stages upon the development of the grain has been clarified, and also the behavior of carbohydrate under the various environmental conditions has been studied.

Generally speaking, the starch content in the grain should be controlled mainly by the photosynthetic activity in the green parts after flowering, and by the amount of the carbohydrate stored in the stem.

The carbohydrate in the stem, which could be translocated to the ear from the stem, depends upon the carbon dioxide fixed in the leaves before the flowering. Being interested in the mechanism of such movement of carbohydrates from the standpoint of carbon metabolism at the time of maturity of the cereal crops, experiments were made on the translocation of the substances assimilated in the leaves before and after the flowering. In this paper, at first, the results obtained on the behavior of the carbohydrate in the rice plant at the stage of the grain maturity after the pollination will be described. Recently, numerous experiments on the carbohydrate metabolism in the plant have been carried on by applying C^{14}, the long lived isotope of carbon, and it has been proved that C^{14} is one of the most useful tracer isotopes in biological research (5, 6, 7, 8, 9). As this technique, especially the radioautography of C^{14} is expected to be effective in our study, this method was adopted.

Material and Method

The seedlings of the rice plant, Norin No. 16, were transplanted on June 10, 1956, and carefully cultured in porcelaine pots filled with alluvial soil by the usual method. They flowered on September 13. On the 20th day after flowering, they had still four green living leaves. Half of the 12th leaf, namely the lowest of these living leaves and the tip of the 13 th leaf were yellow but the others were green. From this state, it was indisputable that the leaves except the 12 th still maintained the ability of the photosynthesis. The carbon dioxide labelled with C^{14} were incorporated into each of the upper leaves, that is, 15th, 14th and 13th leaves.

The apparatus for incorporating $C^{14}O_2$ to the each leaf is shown in Fig. 1. The leaf is covered with a glass tube (*1*), the mouth of which is sealed by a rubber plug (*2*), which has been cut into two. The leaf is placed between the two pieces of the rubber plug. The rubber plug is covered with a small amount of water, acidified with hydrochloric acid for preventing the leakage of the gas. The gas generator is illustrated by (*4*). A certain volume of sodium carbonate solution labelled with C^{14} is taken in (*a*) by means of the injector. The cock (*b*) is closed and a certain amount of acid is poured in (*c*). The tube of the soda lime is attached to (*c*) as shown in (*d*). The cock (*6*) is opened and the air in the vessel (*a*) and the glass tube (*1*) is sucked up by the suction pump through the exit cock (*6*) Then, the cock (*6*) is closed. The cock (*b*) is opened to pour the acid into (*a*). $C^{14}O_2$ will be expelled from the sodium carbonate solution and flow into the glass tube. The leaf is illuminated by the light of 500 W reflector lamp (*3*). After a certain time, the air including $C^{14}O_2$ is sucked up through the cock (*6*) and absorbed in the washer containing barita solution (*5*). On the state of the suction, the cock (*b*) is opened and fresh air is passed through the glass tube.

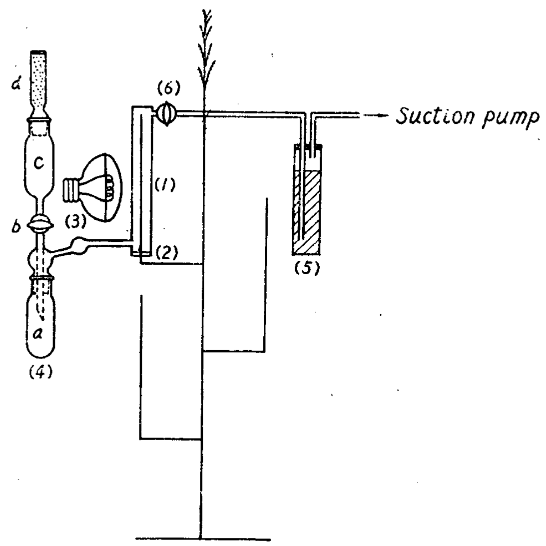

Fig. 1. Diagram of the apparatus for the incorporationof $C^{14}O_2$ into the leaf blade.

At the end of the experiment, the sample is taken out and separated into the individual organs, the ear, internodes, leaf blades and leaf sheathes. The grains are removed from the ear. By pressing the organs with a flat iron, the enzymatic action of the organs is stopped and the materials are flattened. They are arranged on a plate and brought into contact with a X-ray film. The plate is left in the dark room for 7 to 14 days. Thereafter it is developed and printed.

Results and Discussion

When C^{14} was incorporated into the blade of the boot leaf, 15th leaf, only a part of C^{14} moved to the node to which that leaf was attached, most of its activity remaining in the leaf blade. Then the flow of C^{14} took two routes, namely to the upper and lower parts, from this node. C^{14} moved towards the upper direction distributed in the whole of the ear uniformly, or C^{14} translocated to the peduncle within 6 hours through the sheath and the node of the same leaf. It was recognized that the translocated materials arrived at the middle parts of the rachis within 12 hours and at the top of the ear within 24 hours (Figs. 2, 3). The flow of C^{14} towards the lower part made a short stop at the next node. After that, it went farther to the lower nodes. In Figs. 2, 4 and 5 it is shown that C^{14} is distributed uniformly throughout the internode but there is a marked difference in the content of C^{14} between the node and the internode. While, through the node a quite small amount of C^{14} entered the leaf sheath under the boot leaf, but could not reach the leaf blade. So, there may be no flow of carbon from the boot leaf blade to the leaf blades underpositioned. To remove the difficulties of the flowing, the lower leaves were kept in dark for exhausting the stored carbohydrates and only the boot leaf was exposured to the light for accumulating carbohydrates, because the fall of the carbohydrates between the boot leaf and the others would be increased by this method. However, in spite of this treatment, no movement of carbon from the boot leaf to the lower leaves was observed.

When C^{14} was incorporated into the next leaf, that is, 14th leaf, the distribution of C^{14} was quite different from that incorporated into the boot leaf. In this case, C^{14} incorporated into this leaf blade never reached to the ear and stopped at the node of the boot leaf (Fig. 4). Even in eight days after the incorporation into this leaf, C^{14} was not recognized in the ear and the other leaves, the boot leaf and the lower leaves.

The same phenomenon was observed also in the case of the incorporation into the third leaf under the boot leaf, that is, 13th leaf (Fig. 5). Generally, C^{14} incorporated into a certain leaf was not able to enter the other leaf in this stage of the rice plant, but it was clear that C^{14} was distributed in the tiller which grew from the same node of the incorporated leaf.

Each of these experiments was carried on the former half in the light the latter half in the dark. The same results were obtained also in the experiments under the continuous illumination by 500 W reflector lamp.

The kind of organs of the rice plant to receive the materials formed during the course of photosynthesis in the eath leaf blade depends upon some internal conditions. For example, when the premature grains were previously taken out from the ear, C^{14} incorporated in the boot leaf stopped at the peduncle and failed to reach the rachis.

Therefore, on the contrary, when the grains are attached to the ear and retained the normal action of carbohydrate accumulation the ear will be able to receive the photosynthetic products. As mentioned above, photosynthetic products in a certain leaf are distributed into organs in a definite order. As to this phenomenon, there is a question why it is difficult to enter into the ear from the lower leaves in spite of the easy movement of C^{14} into the ear from the boot leaf. For this question, first, it is considered that this phenomenon was caused by the difference between the age of the boot leaf and the lower leaves. In the nitrogen metabolism, it is known that each leaf, during its life cycle, passes three stages—first anabolic, second stable and third catabolic stages. From the standpoint of the carbon metabolism, perhaps the following stages may be established: the first anabolic stage corresponds to a period when a leaf takes up organic materials from other organs, the second stable stage corresponds to a period when a leaf sends out the photosynthetic products from it to the others, that is a period of the height of carbon assimilation and the third catabolic stage corresponds to a period when a leaf itself consumes all the materials produced in its own leaf. In this experimental period, as to the stage of maturity, the boot leaf was in the second stable stage and the lower leaves were in the stage on the way from the second to the third stage. Therefore, it is considered that such a phenomenon was observed. So that, it may be expected that at the earlier stage just after flowering, C^{14} incorporated into the 13 th and 14 th leaves, would move to the ear. While, it is believed that the distribution of the carbon between two organs is controlled by the balance of the concentration in each organ of the 85 per cent ethanol soluble materials, mainly sugars. But the orientation of the flow of C^{14} incorporated into a leaf was decided by not only the fall of the ethanol soluble fraction between the C^{14} incorporated leaf and other organs, but other factors also participate in it. As shown above, C^{14} was not always translocated from the illuminated organ into the other parts which were covered with the black cloth for keeping dark to drop the concentration of the carbohydrates. For the purpose of the interpretation of this matter, we have to consider the next two factors. The first is concerned with the distance between the place from which C^{14} is sent out and the place to accept C^{14}. Now, imagine the case in which C^{14} incorporated into a certain leaf is distributed into two organs which have equal concentration of sugars. It cannot be possible that C^{14} is distributed equally in two organs, even if the above conditions were sufficiently satisfied. Of course, the distance between the leaf and the organ is narrower, the more C^{14} is distributed. Because, C^{14} is consumed as substrates of respiration or is stored by the organs on the way to an acceptor and therefore its concentration decreases when it comes nearer to the receiving organs.

The second factor is concerned in the physiological activity of a receiving organ, viz, the force to suck up the photosynthetic products from other organs. Now, consider the two receiving organs which have same distances from the leaf blade incorporated C^{14} and the conditions on the way are also equall. In this case, C^{14} incorporated, will flow naturally into the direction of the organ having higher activity. Because, it is considered that by the high activity of phosphorylase and other enzymes in the organ the alcohol soluble substances will be changed into other insoluble substances as soon as they came into the grain and, so, the concentration of sugars in the organ will be decreased and concentration gradient of C^{14} between the two organs will be kept to a certain degree. This consideration is applicable in many cases but it is not always so in every cases. If the consideration is completely reasonable, the concentration of sugars must decrease as the sugars progress to an acceptor. But, it was not impossible that more sugars existed in such an acceptor as the grain than in the supplier (10). Then, in that case, C^{14} flowed in opposite directions against the gradient of the sugar concentration. For the present, we have no other suitable interpretation for this phenomenon than these two; the high physiological activity in the receiving organ and the irreversibility of the flow of sugars by the special structure of the receiving organ.

Summary

1. The carbon dioxide labelled with C^{14} was incorporated into each leaf of a rice plant in the maturity stage. The distribution of C^{14} was investigated by the radioautography method.
2. As the result of our experiments, it was recognized that C^{14} incorporated into the boot leaf translocated into the ear within 12hrs. But it was not recognized that C^{14} incorporated into the lower leaves translocated into the ear within eight days.
3. Generally, C^{14} incorporated into a certain leaf was unable to enter the other leaf in this growing stage, but it was clear that C^{14} distributed in the tiller which grew from the same node of the incorporated leaf.

4. The mechanism of these movements of C^{14} is discussed.

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Fig. 2. Radioautographs showing the distribution of C^{14} in a rice plant following initial absorption by the boot leaf blade. Right, radioautograph of treated plant; left, photograph of it. Right to left; ear, peduncle, boot leaf sheath, boot leaf blade, youngest internode, 14 th leaf blade and 14th leaf sheath.

Fig. 3. Radioautographs showing the distribution of C^{14} in ears following initial absorption by the boot leaf blade. Right, radioautograph of treated plant; left, photograph of it. Right to left, 72 hrs, 24 hrs and 12 hrs after incorporation.

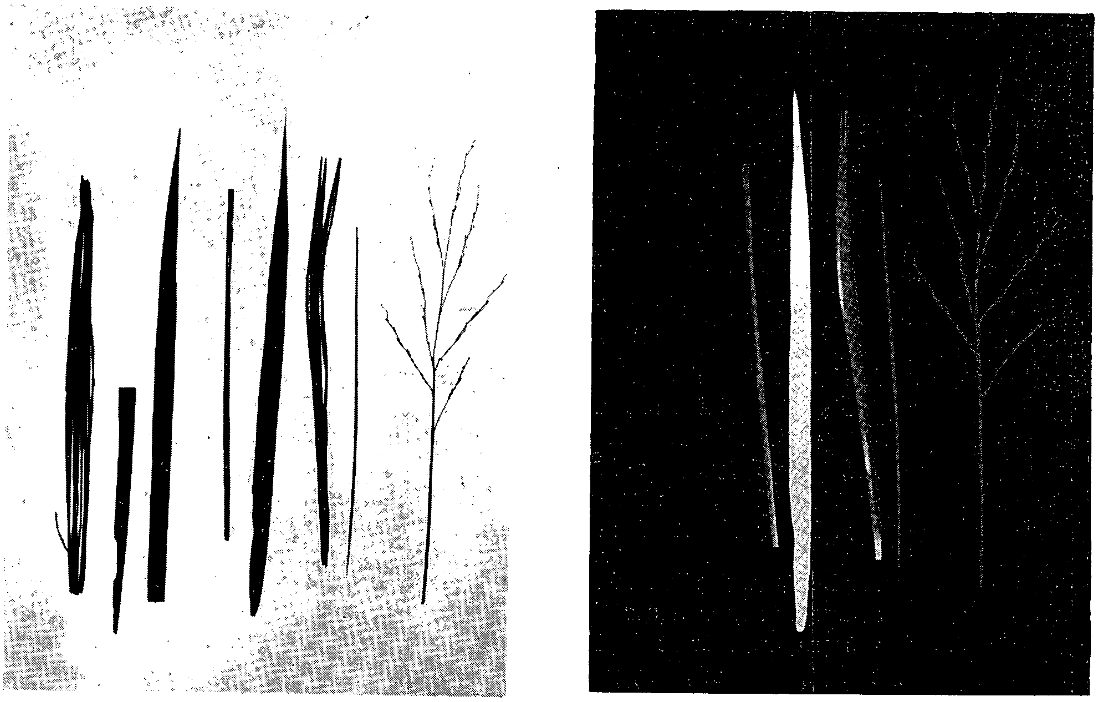

Fig. 2.

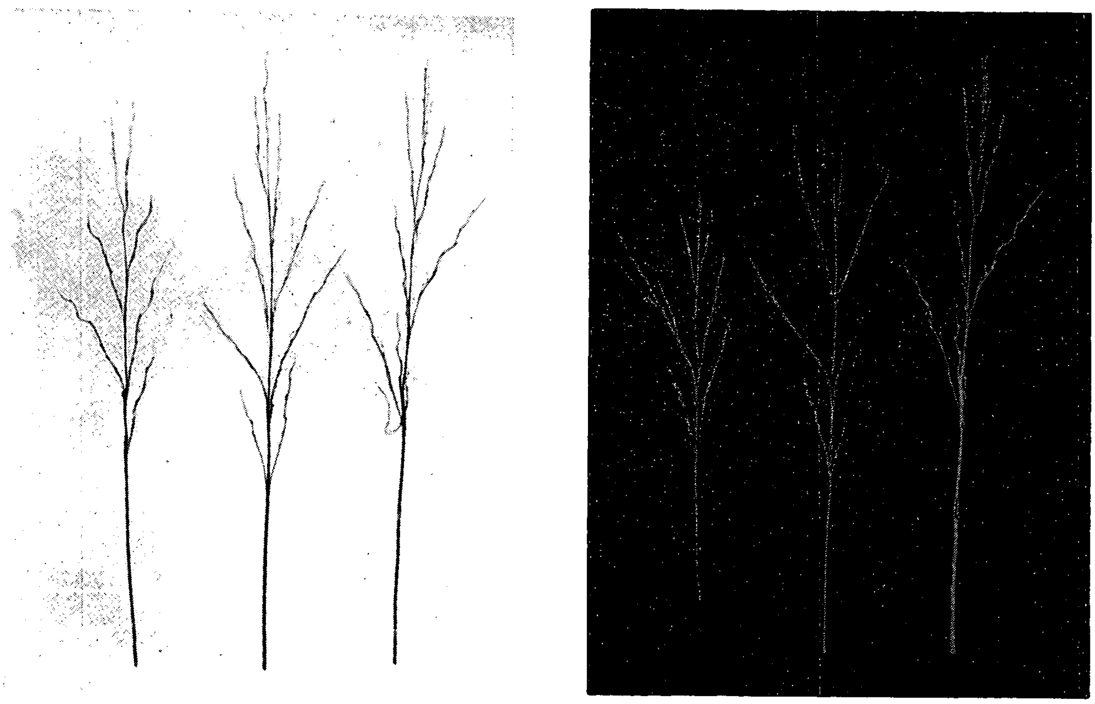

Fig. 3.

Fig. 4. Radioautographs showing the distribution of C^{14} in a rice plant following initial absorption by the 14th leaf blade. Right, radioautograph of treated plant ; left, photograph of it. Right to left, ear, peduncle, youngest intermode, 2nd and 3rd youngest internodes, boot leaf blade, boot leaf sheath, 14 th leaf blade, 14 th leaf sheath, 13th leaf blade and 13th leaf sheath.

Fig. 5. Radioautographs showing the distribution of C^{14} in a plant following initial absorption by the 13th leaf blade. Right, radioautograph of treated plants ; left, photograph of them. Right to left, peduncle, ear, youngest internode, 2nd and 3rd youngest internodes, boot leaf sheath, boot leaf blade, 14 th leaf sheath, tiller of 14th leaf, 14th leaf blade, 13th leaf sheath, tiller of 13th leaf, 13th leaf blade, 12th leaf sheath and 12th leaf blade.

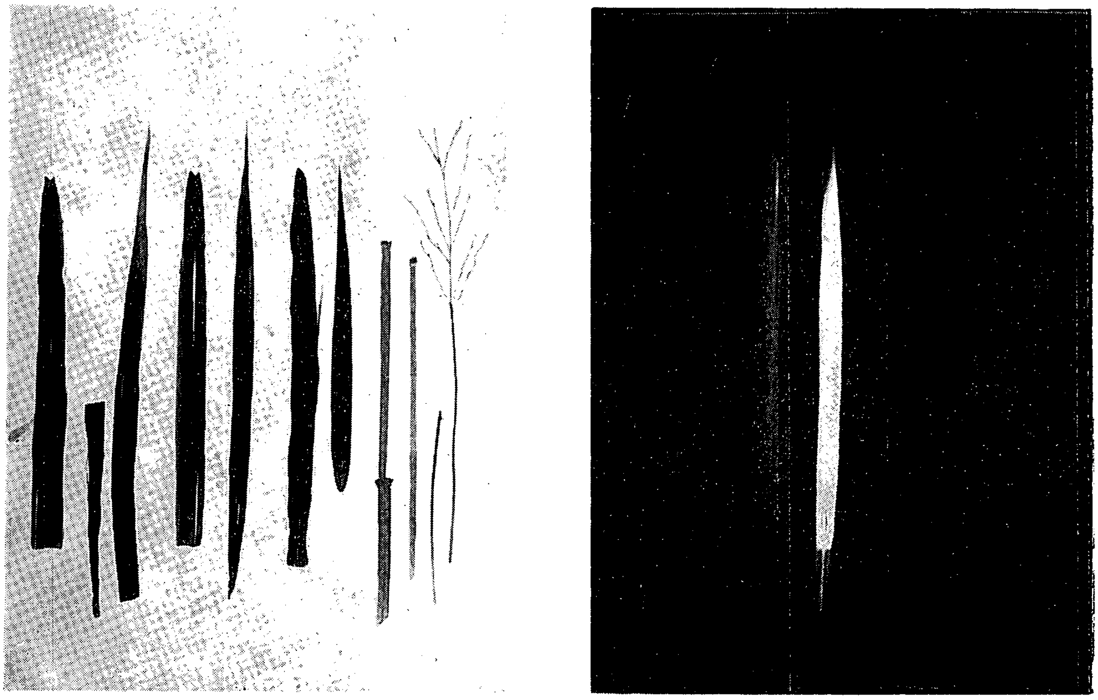

Fig. 4.

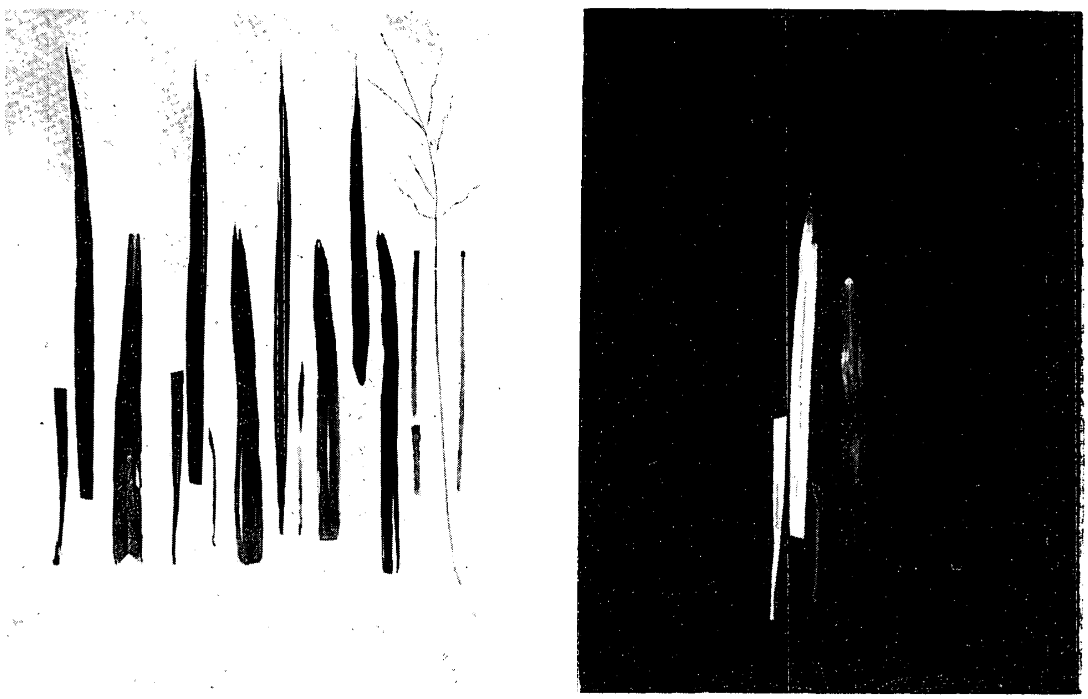

Fig. 5.