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Radioisotopic Study of Chlorophyll Accumulation in Soybean Leaves, in the Conditions of a Transfer from one Daylength to another ("Transfer-Effect")

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Introduction

Chlorophyll in the leaves is easily labeled through biosynthesis (Roux and Husson 1952, Shlyk and Godniev 1958, Brzeski and Rüker 1960); glycine-C^{14} and Δ-aminolevulinic acid-C^{14} are particularly efficient precursors.

It has been also shown that the accumulation chlorophyll does not follow exactly the same pattern when a given species is grown in short days instead of long days (Cheuvart 1954, Clauss and Rau 1956, Sironval 1957 a and b, Enloe 1959). This is particularly true for plants sensitive to photoperiodism. In this case, drastic changes in the pattern of accumulation of the green pigments can be obtained when the plant is transfered from one to another daylength at definite developmental stages. It is the "transfer-effect" which has been observed in Fragaria vesca (Sironval 1957 b) in Cannabis sativa (Cheuvart 1954) and in Soja hispida (Sironval 1957 a).

It appeared interesting to investigate this "transfer-effect" with isotopes and see what it could reveal of the biochemical relationship between the chlorophylls a and b.

Material and Methods

We use *Soja hispida,* variety Capitole. The plants are grown in two series — in short days (8 hrs) and long days (16 hrs) — at 20°C constant under artificial light of fluorescence Phytor lamps (about 5000 lux) from the seed to the appearance of the third leaf. At this time, one half of the plants grown in long days is transfered to short days, and one half of the plants grown in short days is transfered to long days. We obtain in this way four series:

1. Soybeans grown in long days, remaining in long days;
2. Soybeans grown in long days, transfered to short days;
3. Soybeans grown in short days, remaining in short days;
4. Soybeans grown in short days, transfered to long days.

Just at the moment of the transfer, all the leaves except the very young third leaf are removed. The removal of the leaves is done in such a way that the midrib and the petiole of the second leaf are left on the plant. This petiole is dipped during 24 hrs in a solution of Δ-aminolevulinic acid-4-C^{14} (10 mg. or 200 μC/100 ml. solution).

The content of chlorophylls and the labeling of the two forms a and b is followed during the growth to the senescence of the third leaf (about 30 days) in the four sets of plants. The chlorophylls are extracted in acetone and the total quantity calculated following Mackinney (1941). An aliquot of the acetone extract is chromatographed on paper following Sironval (1954) and the chlorophylls a and b are eluted separately in ether. Chromatography according to Chiba and Noguchi (1954) has been performed several times in order to obtain a proper purification of the pigments. The quantities of the pigments in the eluates are measured following Comar (1942). The radioactivity is measured in a flow counter Tracerlab SC-16 working in the Geiger region.

Results

Figure 1 shows the accumulation of the total chlorophyll (a+b) per g. of fresh weight in the four series. It is seen that the transfer from long to short days depresses the accumulation in the early stage of growth of the leaf, whereas the transfer from short to long days accelerates the accumulation.

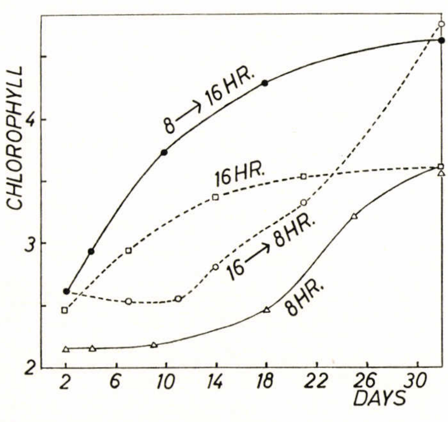

Figure 1. *Course of the accumulation of total chlorophyll* (a+b) *in the four experimented series* (expressed in mg. total chlorophyll per g. fresh weight of the third soybean leaf). Daylength given in the diagram.

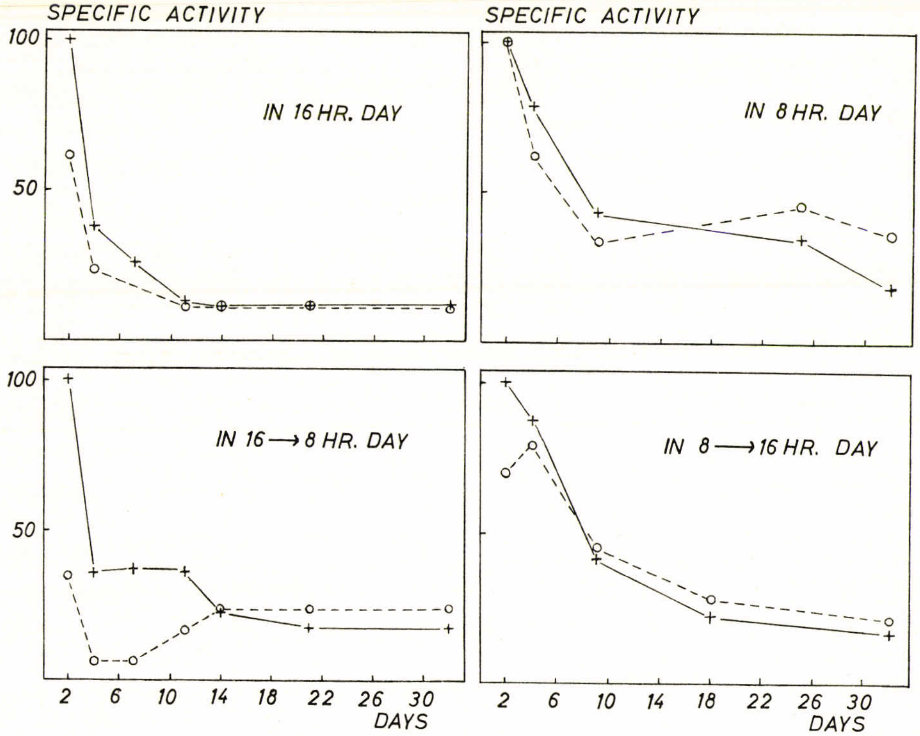

Figure 2. *Evolution of the specific activity of the two chlorophylls a and b during the growth of the third soybean leaf in the four experimented series* (expressed in relative units; 100=the specific activity of chlorophyll a the second day of the experiment).

The series transfered from short to long days accumulates much more chlorophyll than the series grown in long days permanently from the sowing. It is interesting to note that this "transfer effect" is obtained in very young leaves, in the absence of any adult leaves which have been removed just at the moment of the transfer. The very young leaves appear to be very sensitive to the transfer from one to another daylength.

Figure 2 gives the evolution of the labeling of the two chlorophylls a and b expressed as specific activities in relative units. A maximum of radioactivity is always reached two days after the beginning of the application of Δ-aminolevulinic acid; thereafter, the specific activity decreases rapidly. On the 10th to 15th day, it has reached a low level where it remains approximately constant until the end of the experiment, 10—15 days later.

The first 10—15 day period corresponds to the rapid growth and extension of the young leaf. During this period, the two pigments accumulate more

Table 1. *Variations of the ratio* $\frac{\text{specific radioactivity of chlorophyll a}}{\text{specific radioactivity of chlorophyll b}}$ *in the four experimented daylength treatments.*

Time in days	Series			
	16 hr-day	8 → 16 hr-day	8 hr-day	16 → 8 hr-day
2	2,0	1,4	1,0	2,8
4	1,6	1,1	1,2	6,2
6	1,4	0,9	1,3	8,0
20	1,0	0,8	0,9	0,8

or less rapidly in the leaf in the four experimental series. (Figure 1 expresses the quantity of pigments per g. fresh weight. When this quantity remains constant in the young leaf, this does not mean that there is no net pigment accumulation, since the fresh weight increases!) Therefore the observed decrease of the activity is certainly at least partially due to a dilution. It is difficult to evaluate the role played in the process by the pigment turnover.

The second 15—20 day period corresponds approximately to the expanded adult leaves. During this period the constancy of the label indicates a rather high stability of the two pigments in the chloroplasts.

During the first six days of the experiment, chlorophyll a is generally found more labeled than chlorophyll b. Table 1 gives the ratio $\frac{\text{specific radioactivity of chlorophyll a}}{\text{specific radioactivity of chlorophyll b}}$ $\left(\text{ratio } \frac{a^*}{b^*}\right)$ two, four, six, and twenty days after the application of Λ-aminolevulinic acid. It is seen that during the first six days of the experiment, the ratio $\frac{a^*}{b^*}$ reaches values equal to 1.0 or under 1.0 in two cases only. These lowest ratios are found in the series which have been grown under short days before the application of Λ-aminolevulinic acid. (8 hr-day and 8 hr → 16 hr-day series). The ratios of the series previously grown under long days are always higher (16 hr-day and 16 hr → 8 hr-day series). The highest ratios are found in the series which has first been grown under long days and has been transfered to short days (16 hr → 8 hr-day series). Here exceptional values up to 8.0 are recorded. This indicates that the photoperiodic treatment of the plants has an effect on the relative labeling of the two chlorophylls a and b, as it has on the accumulation of the pigments in the growing leaf.

Fifteen days after the beginning of the experiment, the labeling of chlorophyll b is equal or a little higher than that of chlorophyll a (see Figure 2 and table 1 after 20 days). The picture is very different from that of the first six days. In particular the attentive consideration of the series transfered from 16 hr to 8 hr-day (Figure 2) shows that the evolution of the ratio from high values during the first days of the experiment to low values at the end of the experiment, occurs from the 7th to the 14th day by a simultaneous increase of the specific activity of chlorophyll b and decrease of the specific activity of chlorophyll a. This particular case may suggest a formation of some molecules of chlorophyll b from molecules of chlorophyll a.

Discussion

It is clear from the results that the accumulation of the two chlorophylls a and b in the soybean third leaf is influenced by the photoperiodic treatment given to the plants. This is not suprising since such effects are known for different plant species (Sironval 1957 a). We intend only to insist on the two following points:

1) The "transfer effect" is obtained in the young growing third leaf in the absence of any adult leaves. This means that the young leaf "remember" the conditions in which the previous adult leaves have been grown. When the plants are transfered from short to long days for instance, the young leaf "feels" the previous short day treatment: the transfered growing leaf accumulates much more pigments than the control leaf which has been permanently grown in long days. In this case, *it seems that something accumulates in short days which is able to increase the chlorophyll synthesis after the transfer to long days.*

2) The use of Λ-aminolevulinic acid labeled with C^{14} shows that, in the very young growing leaf, the relative labeling of the two chlorophylls a and b can vary according to the daylength treatment. In the young leaf the ratio $\frac{a^*}{b^*}$ is lower when the plants have first been grown in 8 hr-day than when the plants have first been grown in 16 hr-day. Some results seem to indicate a possible formation of some chlorophyll b molecules from chlorophyll a molecules. However, if it exists, such a process must be very slow: a rapid interconversion is excluded, since in our experiments the equalisation of the labeling of the two pigments requires several days; *it is thus very doubtful that the reversible transformation chlorophyll a $\rightleftarrows$ chlorophyll b — if it exists — plays any role in the dissociation of water molecules involved in photosynthesis* (see also Blass, Anderson and Calvin 1959).

A possible way to account for a slow transformation a → b consists in the assumption that the conversion of a molecule of a to a molecule of b could occur from time to time in the chloroplast, for instance as a result of an occasional and local photoxidative process (depending on the local conditions of protection, light intensity, etc.). In fact, photoxidative experiments do not exclude the possibility that during photoxidation there is some production of chlorophyll b as a result of the oxidation of chlorophyll a (Sironval and Kandler 1958).

In our experiments photoxidation could be more or less easy according to the daylength treatment. For instance it is possible that the chloroplasts of the plants previously grown in long days have an internal organisation which protects the chlorophylls from photoxidation better than that of the chloroplasts previously grown in short days. In this case, photoxidation would be lower — and therefore the ratio $\frac{a^*}{b^*}$ higher — in the series first grown in long days, what we effectively find. A particular case would be that of the series grown in long days and transfered to short days. Here the internal protection to photoxidation would be good and the short duration of the light period after the transfer would not allow much photoxidation. In fact we find, in this case, exceptionally high $\frac{a^*}{b^*}$ ratios in full agreement with the suggested hypothesis.

Summary

The transfer of Soybean plants from one daylength to another greatly affects the accumulation of the chlorophylls in the leaves. This "transfer-effect" is studied using C^{14} Δ-aminolevulinic acid as tracer. It is shown that the relative labeling of the two forms of chlorophyll (a and b) varies according to the daylength treatment. A tentative interpretation of the facts is proposed. It is based on the assumption that in certain conditions, some molecules of chlorophyll b could be produced as a result of the photoxidation of some molecules of chlorophyll a.

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