Physiological Studies on the Outgrowth of the Epicotyl in 
Stizolobium hassjoo V. Changes in Respiratory 
Metabolism in the Cut Region 
of Etiolated Epicotyl

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Abstract

Changes in respiratory metabolism in the cut region, a region 0–2 mm below the 
cut surface, of the decapitated epicotyl of etiolated Stizolobium hassjoo seedling 
were followed under the dark and light conditions; under the former conditions 
the callus formed on the cut surface, under the latter not. The following results 
were obtained. 1) The rate of \(^{14}\)CO\(_2\) output from G-U-\(^{14}\)C increased after cutting 
both in the dark and in the light, but more notably under the dark conditions. 2) 
In the dark, malonic inhibition rate of CO\(_2\) release from glucose decreased remark-
ably in a day after cutting, while in the light, such a drastic change was not 
oberved. 3) \(C_6/C_1\) ratio rapidly fell in a day after cutting in the dark. In the 
light the change in the ratio was not so striking. From these results it is suggested 
that under the dark conditions, drastic changes in metabolic activity occur in the 
cut region in a day after cutting, when any notable morphological change can not 
yet be observed, and that the activity of the pentose phosphate pathway consider-
ably increases there.

As already reported\(^1\), when the epicotyl of etiolated seedling of Stizolobium 
hassjoo, ca. 10 cm long, is cut at the position more than 3–4 cm distant from the tip, 
a callus remarkably outgrows from the cut surface. It has been shown\(^2\) that in the 
callus, the contribution of the pentose phosphate (PP) pathway to glucose catabolism 
is much greater as compared with that in the part of the epicotyl, where the callus 
derives.

For investigating the mechanism of the callus formation, it is important to know 
the metabolic processes through which cell division in callus development is induced. 
In this study, the changes in the respiratory metabolism in the cut region of the 
epicotyl were followed with time after cutting.

Material and Methods

Material: The plant material used in this study was the same as that described 
in previous papers\(^1\)–\(^4\). The seeds of Stizolobium hassjoo were sown in quartz sand 
without any nutrient and kept at 27–30° in the dark. When the epicotyls of etiolated 
seedlings grew to be 10–12 cm long (about a week after sowing), they were decapi-
tated at the position 5 cm distant from the tip and the seedlings kept in the dark

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or in the light (fluorescent lamp, 3,600 lux).

All experiments in this work were carried out with slices. A part of the epicotyl 0-2 mm under the cut surface was named "cut region" and two successive 1 mm sections were transversely taken from this part, and each section was separately used for experiments. In addition, 1 mm slices from the part VII were also used as a control. Various parts of an epicotyl were designated as shown in Fig. 1.

**Nitrogen determination:** Total and protein nitrogen contents were determined by the same methods as in the previous paper.

**Measurements of respiratory gas exchange:** The output of $^{14}$CO$_2$ from $^{14}$C-labeled glucose supplied to slices was examined in the following manner. In a flask with a center well, about 30 sections of slices were put together with 7 ml of phosphate buffer (10$^{-2}$ M, pH 6) and 3 ml of radioactive glucose solution (0.15 μc, about 10$^{-3}$ mg) were added. The flask was shaken at 30°C for 2-2.5 hours in the dark. When malonic acid was used as a respiratory inhibitor, it was dissolved in the buffer and the pH adjusted to 6 with NaOH. The final concentration of malonic acid was 5× 10$^{-2}$ M. Respiratory CO$_2$ containing $^{14}$CO$_2$ was trapped with NaOH in the center well of the flask. The radioactivity of trapped $^{14}$CO$_2$ was estimated as usual.

**Results**

**Effect of the light on the callus formation:** When the etiolated seedling of Stizolobium hassjoo is decapitated at the position 5 cm distant from the tip and further kept under the dark conditions, a callus readily develops. But when the seedling is transferred to the light after cutting, the callus is not formed. As a preliminary experiment to investigate the light effects on the callus formation, the seedlings grown in the dark and decapitated as above were transferred to the light of various colors, blue (400-500 mμ), green (500-600 mμ) and red (600-700 mμ), and it was examined whether the callus was formed on the cut surface in any color light, as in the dark. The results are summarized in Table 1. In any color light, the callus was not formed after a week, during which the callus formation in the dark was readily detected. After two weeks, only

![Fig. 1. Designation of various parts of an epicotyl of the etiolated Stizolobium hassjoo seedling.](image)

| Table 1. Effect of light* of various color wave-lengths** on the callus formation. |
|---------------------------------|--------|--------|--------|--------|--------|
|                                 | Daylight | Blue | Green | Red | Dark |
| 1 week after cutting            | -       | -     | -     | -    | +     |
| 2 weeks after cutting           | -       | -     | -     | ±    | +     |

* Energy: 0.02-0.03 cal/min · cm².

** Daylight: daylight fluorescent lamp, Blue: Mitsubishi colored lamp FL-40-BF, Green: FL-40-GF, Red: FL-40-RF.
+ : Callus formed, ± : formed in low frequency, - : not formed.
in the red light experiment, a callus formation was observed. But the frequency of the callus formation was very low (20-30%), as compared with that observed in the dark (90-100%), and the appearance of the callus formed in the red light was quite different from that of the typical one formed in the dark. The latter was whity and developed into a gall-like mass in two weeks, while the former was very small, hard and blackish.

Anatomical observation: Anatomical changes occurring at the cut region under the dark conditions were followed with time after cutting. In Figs. 2-5 are shown longisections of the cut region of four stages (0, 1, 4 and 7 days after cutting). Any notable anatomical changes are not seen in a day after cutting, while 4 days after cutting a remarkably active cell division is observed in cambial zone at the cut region. Just corresponding to this activity, a prominent thickening is observed from the cut surface to the depth of about 2 mm. From Figs. 4 and 5, it can be seen that the callus originates in cambium and its adjacent parenchymatous cells.

Changes in dry weight: Under the dark conditions, dry weight per slice of the upper region increased considerably during a day after cutting and gradually thereafter, while under the light conditions, the upper region did not show such a marked increase in dry weight (Fig. 6). Dry weight of the part VII remained almost unchanged in the dark during the experimental period. In the light, on the other hand, there was observed a gradual increase in dry weight through the experimental period in any part tested.

Nitrogen contents: In Fig. 7 are shown the changes in the contents of total and protein nitrogen in the three parts of the epicotyl under the dark conditions. The variations in the nitrogen level per dry weight were very striking in the upper and the lower region. In the upper region, there was a marked drop in the amount of
total nitrogen on the first day after cutting and subsequently a gradual return to the initial level. The protein nitrogen content in the upper region, however, increased a little in a day after cutting and retained the level during the experimental period. In the lower region, on the other hand, the total nitrogen content per dry weight rose notably in a day after cutting and gradually fell to the initial level thereafter. The protein nitrogen content in the lower region increased a little in a day after cutting, but afterward it returned to its initial level. When expressed on slice basis, the total and protein nitrogen contents of both the upper and the lower regions increased markedly during the first day. Both the total and the protein nitrogen contents in the part VII were kept almost at the same level through the experimental period on both bases.

Respiratory activity: Under the dark conditions, the relative activity of $^{14}$CO$_2$ output from uniformly labeled glucose (G-U-14C) among the three parts was similar on both dry weight and slice basis (Fig. 8). The respiratory activity of the upper region was the highest throughout the experimental period. It increased remarkably during the first 3 days and the level was kept thereafter. The rate of $^{14}$CO$_2$ release in the lower region also increased during the first 3 days but not so notably as in the upper region. The activity of the part VII was the lowest and little changed during the period. Under the light conditions, the relative activity of $^{14}$CO$_2$ output from uniformly labeled glucose (G-U-14C) among the three parts was similar on both dry weight and slice basis (Fig. 8).
conditions, on the other hand, the rate of $^{14}$CO$_2$ output increased in all the three parts during the first several days, although there was a little difference in the activity among the three. The increment of the $^{14}$CO$_2$ output rate on dry weight basis in a day after cutting was not so different in the two experiments, in the dark and in the light. But the rate observed under the dark conditions increased more remarkably thereafter than that under the light conditions.

The $^{14}$CO$_2$ output rates of the upper region kept in the dark and light were compared with those in the presence of malonic acid and the results are shown in Fig. 9.

![Fig. 8. $^{14}$CO$_2$ output from G-U-$^{14}$C in the cut region and the part VII of the epicotyl kept under the dark and light conditions. •: upper region, ○: lower region, □: part VII.](image)

![Fig. 9. $^{14}$CO$_2$ output from G-U-$^{14}$C in the presence and absence of malonic acid in the upper region of the epicotyl kept under the dark and light conditions after cutting. •: control, ⊱: +malonic acid.](image)
Malonic inhibition rate of CO₂ output: Malonic inhibition rates of ¹⁴CO₂ output from G-U-¹⁴C in the three parts were examined and the results are shown in Fig. 10. Under the dark conditions, the inhibition rate of the upper region was very high (80%) immediately after cutting, but during a day after cutting, it decreased remarkably (from 80 to 20%). That of the lower region also decreased during a day after cutting but not so markedly as that of the upper region. The part VII showed an unaltered inhibition rate during the experimental period. Under the light conditions, however, such changes as seen in the dark were not observed during the first day. A gradual decrease in the inhibition rate was seen through 7 days in every part, though a little difference in the decrease rate was observed among the three.

C₆/C₁ ratio: Changes in the C₆/C₁ ratio were followed with time after cutting. As shown in Fig. 11, a great difference in changes in the ratio was observed between the dark and the light experiment. One of the most noteworthy points was that a drastic change in the ratio was seen during the first day after cutting as in the malonic inhibition rate. The C₆/C₁ ratio of the upper region under the dark conditions fell remarkably during the first day, while under the light conditions slightly.

Fig. 10. Changes in malonic inhibition rate of ¹⁴CO₂ output from G-U-¹⁴C in the cut region and the part VII of the epicotyl kept under the dark and light conditions after cutting. ●: upper region, ○: lower region, O: part VII.

Fig. 11. Changes in C₆/C₁ ratio in the cut region and the part VII of the epicotyl kept under the dark and light conditions after cutting. ●: upper region, ○: lower region, O: part VII.
In the dark, the ratio of the lower region fairly decreased, and that of the part VII remained unchanged. In the light, the change in the ratio was not so striking and the ratio had a slight tendency to decrease in all the three parts.

**Discussion**

The callus formation in the epicotyl of etiolated *Stizolobium hassjoo* seedling readily occurs when kept in the dark after cutting, but the callus is not formed in the decapitated seedlings kept in the light. In order to investigate the difference in respiratory metabolism between the cut regions (Fig. 1) from which the callus is formed and not formed, the comparisons were made between the seedlings kept throughout in the dark from their germination and those germinated in the dark and moved to the light after cutting.

Physiological effect of cutting on tissues seems to attain to several millimeters of depth from the cut surface, according to the data obtained from the experiments with potato tubers and *Vicia faba* seedlings. In *Stizolobium* seedlings, the thickening in the cambial zone is observed to reach a depth of about 2 mm (Fig. 4), and in the present work the two successive 1 mm slices from the cut region and slices of part VII as a control were compared one another.

It is very remarkable that dry weight of the upper region considerably increased under the dark conditions in a day after cutting, when any notable anatomical changes could not be found (Figs. 2, 3 and 6). Under the dark conditions, therefore, an active accumulation of dry matters seems to occur in the upper region in a fairly short time after cutting. This may be inferred from a rise in total nitrogen content per slice (Fig. 7). But, on the contrary, total nitrogen content of the upper region, expressed on dry weight basis, considerably decreased in a day after cutting. This might suggest an accumulation of a larger quantity of dry matters other than nitrogen compounds in the upper region. Gradual increase in dry weight in the three parts under the light conditions would result, at least partly, from light effects, that is, in the light, tissues gradually become green, substantial and structurally strong.

Under the dark conditions, protein seems to be very actively synthesized in the upper region, and also in the lower region though in a limited degree, during a day after cutting (Fig. 7). As shown in Figs. 2, 3 and 5, cell division is seldom or not observed in the cut region at the beginning but occurs actively during 1-3 days after cutting. It is noteworthy that active protein synthesis occurs in the cut region before rapid cell division is observed. On investigating the changes in nitrogen content and in respiration rate of *Acer pseudoplatanus* cells in suspension culture, Givan and Collin reported that cells may show high rates of protein synthesis and respiration during the period when they are being prepared for division.

To get a general view of changes in glucose metabolism, malonic effect on respiratory rate was investigated as the first step. One of the noteworthy points in the results of the changes in malonic inhibition rates of $^{14}$CO$_2$ output from G–U–$^{14}$C is that under the dark conditions inhibition rate of the upper region sharply declined in a day, while under light conditions continued to decrease slowly through the experimental period (Fig. 10).

Respiratory rate of the upper region considerably increased after cutting under both the light and the dark conditions (Fig. 8). As seen from Fig. 9, the increase in respiratory rate under the dark conditions results almost from the increase in the activity of the malonate insensitive pathway, and under the light conditions results
from that in the activity of both the malonate sensitive and insensitive pathways. The data (Figs. 9 and 10) on malonic effects indicate that under the dark conditions drastic changes in the participation of respiratory pathways occur, that is, contribution of malonate insensitive route to respiratory metabolism becomes much greater in the upper region during the first day following the cutting.

\[ \frac{C_6}{C_1} \text{ ratio}^{10,11} \] was determined to know the relative participation of the PP pathway. As shown in Fig. 11, under the dark conditions, \[ \frac{C_6}{C_1} \text{ ratio of the upper region} \] sharply decreased during a day after cutting, while under the light conditions not so sharply.

From these results, it is strongly suggested that the malonate insensitive route may consist mainly of the PP pathway and this pathway becomes considerably active in glucose catabolism during the first day after cutting under the dark conditions, under which the callus is formed on the cut surface. In the light where the callus is not formed, such drastic changes in glucose metabolism can not be observed. The possibility may not be ruled out that fermentation, a malonate insensitive process similar to PP pathway, also contributes to glucose breakdown.

Malonic inhibition rate gradually decreases when the etiolated seedlings are transferred to the light (Fig. 10). This is, at least partly, due to the increasing contribution of the PP pathway (Fig. 11). Farkas et al.\textsuperscript{12} reported that the illumination of etiolated wheat seedlings by direct daylight enhances their sensitivity of \( O_2 \) uptake to malonate. This presents a striking contrast with the results obtained with etiolated \textit{Stizolobium} seedlings. Laties and Hoelle\textsuperscript{13} pointed out that failure of potato slice respiration to be depressed by cyanide is due to compensatory induction of another oxidative pathway which maintains the \( O_2 \) uptake at the control level. The \( O_2 \) uptake by sub-apical parts of corn roots, in which the TCA cycle is very active, is largely resistant to malonate. According to Lips et al.\textsuperscript{14}, the resistance of corn roots to malonate is not due to the compensatory mechanism, but due to the utilization of endogenous (cytoplasmic) malate as acetyl acceptor and the resulting conversion to succinate.

It has been reported that in the callus from the cut region the PP pathway plays a fairly important role in glucose catabolism\textsuperscript{2}. It is very suggestive, in this connection, that the activity of the PP pathway becomes remarkably high in the cut region prior to cell division in the cambial zone of that region. However, it is an open question whether the activity of the PP pathway increases exclusively in the cambial zone or in the whole cut region.

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References

8) In preparation.
諸橋哲雄*・鈴嶋 敦*・下郡山正己*：ハッショウマメのめばえにできるカルスの生理学的破壊 V. 上胚軸のカルス形成部位における呼吸系の変動

暗所で育てたハッショウマメのめばえの上胚軸を先端から 5 cm の所で切断すると、すでに報告したように、切断側も引続き芽が発生するため、切断面にカルスが形成される。切断後明所において、切断面が明条件、暗条件、あるいは暗条件で形成される場合（明条件）には、形成されない。この切断面から 2 mm までの切断部でみられる生理的変化を、特に呼吸代謝経路の変動という観点で注目して、明条件と暗条件について作業し、次のような結果を得た。暗条件下では、グルコース-U-14C からの 14CO2 放出速度は、暗条件下で切断後増加するが、特に暗条件下で著しい。

2. 暗条件下では、グルコースからの CO2 放出に対するマルノ酸阻害率は、切断後 1 日で急激に減少するが、明条件下では、そのような激しい変化は認められない。

3. 暗条件下での呼吸の増加は、ほとんどマルノ酸に insensitive な呼吸経路の増加によっているが、明条件下でのそれは、sensitive なものと insensitive なものとの両方によっている。

4. マルノ酸阻害率の変化と平行して、C6/C1 比も暗条件下では切断後 1 日で急激に減少し、明条件下では、それほど顕著な変動はない。

これらの結果から、カルスが形成される暗条件下では、切断後 1 日で切断面に顕著な呼吸系の変動がおこり、ペントースリン酸経路の活性が非常に大きくなるが、カルスが形成されない明条件下では呼吸系の変動は比較的にゆるやかであることがわかる。（* 東京大学理学部植物学教室）