Physiological Studies on Photo-electric Response in Plant Tissues.

II. The Effects of Different Light Intensities on Photo-electric Response of Green Leaves.*

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西崎友一郎**：植物組織の光電反応に関する研究

II. 緑葉の光電反応に及ぼす光の強さの影響

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In a previous paper¹⁾, experimental results were reported on the effects of the duration of light and dark periods before illumination on the photo-electric response in green leaves. It has been shown that the photo-electric response was affected by the duration of the dark period before illumination, and was considerably suppressed by prolonged pretreatment in the dark.

In this paper, the changes in photo-electric response were investigated at different light intensities in relation to the pre-illumination on the leaves.

Material and Method

Green leaves used for the experiments were obtained from the seedling of *Phaseolus vulgaris* L. which had been cultured in a greenhouse for about three or four weeks. The leaflets of the second or third leaf with petiolules were separated from the petiole and immediately immersed in tap water to be kept during the experiments.

The experimental arrangements and the apparatus used were essentially the same as described in the previous paper¹⁾, except for some partial improvements. In the experiments, a leaflet was covered with black paper having a circular hole 2 cm. in diameter through which a part of the leaflet was illuminated. Non-polarizing electrodes of Zn/ZnSO₄ were employed in the measurement of the leaf potential. These were connected through KCl-agar bridges with the starch paste closely in contact with the lower surface of the leaflet at two definite positions, the one under the center of the hole in the black paper and the other under the covered portion.

For the illumination, incandescent light from a projection lamp (100 V, 200W) equipped with a lens system was used. Heat was removed by inserting a filter of a 5 cm.-thick layer of a CuSO₄ solution (6%). By changing the distance between lamp and object on an optical bench, the intensity of the light could be adjusted

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within wide limits. The intensity of the illumination was measured by means of an illuminometer (TOSHIBA, No. 5). The temperature during the experiments was 14-15°.

Experimental

A. Photo-electric response under illumination of different intensities.

A series of experiments was performed to observe the relation between the photo-electric response and the light intensity. It has been shown in previous papers^{1,2)} that the magnitude and the course of the photo-electric response are affected by the length of the dark period before illumination. Furthermore, a pre-illumination preceding a relatively short dark period also changes the ensuing light reactions¹⁾ (see below).

Therefore, experiments were made as follows: the exposed part of the leaflet

Fig. 1. Photo-electric response under different light intensities. Prior to the test-illumination, the leaves were subjected to a pre-illumination of 30 minutes at each light intensity followed by a dark period of five minutes. The upward changes of the curve show that the illuminated portion of the leaflet is negatively charged as compared with the unilluminated portion. The dark period is indicated by the heavy line on the abscissa.

was pre-illuminated with the given light intensities for 30 minutes to obtain a full response by the test-illumination for 10 minutes at the same intensities after the interruption of five minute darkness. The response produced by these test-illuminations was compared with reference to the light intensities of 1,000, 2,000, 4,000, 8,000, 16,000 and 32,000 lux, respectively.

The results are shown in Fig. 1. When the light of 1,000 lux was applied, insignificant variations in the electromotive force were elicited between the illuminated and unilluminated portions. With the light intensity increased to 2,000 lux, two slow negative (upward) peaks were clearly demonstrated. The new small "cusps"^{1,3)}, which first carry the potential difference in the positive (downward) direction before it rises, appeared at light-intensities of 4,000 lux and above. As the light-intensity is increased, the two ensuing negative peaks become increasingly higher and finally fuse into an apparently single peak.

On close investigation of the transition of the negative deflections in the curves in Fig. 1, it is evident that there are essentially two separate components in the negative phase. On illumination at lower light-intensities these two negative components can be distinguished by the difference in time required to reach the respec-

tive peak maxima. When an intense light is applied, however, the maximum of the second peak gradually approaches the first one and finally overlaps it.

B. *Effects of the pre-illumination on the response.*

In the above experiments, the same intensities of light were used in each set of pre- and test-illuminations. Therefore, the amounts of radiation that the leaves received during the pre-illumination varied with different light intensities. The results of the experiments in which different intensities of test-light were applied after pre-illuminating for 30 minutes with light of the 32,000 lux (followed by a dark period of five min.) are shown in Fig. 2 A, B and C.

Both Curve B in Fig. 2 and Curve C in Fig. 1 represent the responses produced by the same test-light of 8,000 lux. It is clear from these curves that the response in the former case, which received a greater amount (or intensity) of radiation in the pre-illumination, is greater than in the latter, which received a lesser amounts (or intensity). The former has an obviously large negative peak while the latter shows two separate peaks.

It is evident from this fact that the photo-electric response increases when the amount (or intensity) of pre-illumination is increased. The same conclusion was derived by comparing Curve C in Fig. 2 with Curve F in Fig. 1.

On the other hand, a typical example of the effect of decrease in pre-illumination on the potential change in intense test-light (32,000 lux) is shown in Curve D as compared with Curve A in Fig. 2. The electrical response in Curve D with 1,000 lux pre-illumination was found to be less prominent as compared with that in Curve A with 32,000 lux pre-illumination; not only the magnitude of the response was decreased but also the second peak appeared as was the case in experiments with lower test-illumination intensity (1,000 lux; Curve C, Fig. 2).

The record of the photo-electric response during a period of intermittent illumination (five minutes light—30,000 lux—and five minutes dark) following a dark period of 30 minutes is shown in Fig. 3. It is clearly shown in this figure that the light response which was insignificant in the first light period became more enhanced on intermittently repeated illumination. At the same time, the two peaks, which appeared in the response curves of the first three periods, fused into a large single peak during the final illumination period.

It is concluded from the above experiments that the light-induced electrical

Fig. 2. Effects of pre-illumination on the photo-electric responses. Prior to the test-illumination, the leaves were kept in darkness for five minutes after the pre-illumination of 32,000 lux for 30 minutes in A, B and C, and of 1,000 lux for 30 minutes in D (Cf., Fig. 1).

Fig. 3. Photo-electric response by intermittent illumination (five minutes light—30,000 lux—and five minutes dark) following a dark period of 30 minutes. The dark period is indicated by the heavy line on the abscissa.

response of the leaves is increased within certain limits by increasing the light intensity of pre-illumination and/or the intensity of the test-light.

C. Effects of varied pre-illumination with a fixed amount of energy.

Further experiments were made to ascertain whether the light-induced photo-

electric response was the same with pre-illuminations with a given energy, but applied at different light-intensities and for different exposure periods. The test-light of 10,000 lux was applied after varied pre-treatment at a constant energy followed by a dark period of five minutes. The results of the five experiments of this series are shown in Fig. 4, in which pre-illumination of 2,000 lux-25 min., 5,000 lux-10 min., 10,000 lux-five min., 20,000 lux-2.5 min. and 50,000 lux-one min. were applied, respectively. It is clear, judging from the comparison of the electrical responses induced by a fixed test-light of 10,000 lux, that the reciprocity law does not hold in so far as the effects of the pre-illumination are concerned. The pre-illumination of 10,000 lux-five min. (Curve C) induced the greatest response to the test-light. As either the power or period of pre-illumination decreased, the corresponding response decreased.

Fig. 4. Effects of pre-illumination with a fixed amount of energy on the photo-electric responses. The leaves were pre-illuminated with the light of 2,000 lux-25 min. (A), 5,000 lux-10 min. (B), 10,000 lux-five min. (C), 20,000 lux-2.5 min. (D), and 50,000 lux-one min. (E), and thereafter kept in the dark for five minutes, prior to the test-illumination at 10,000 lux.

Discussion

The results obtained from the above experiments have an interesting bearing on the nature of the photo-electric response. It is shown that the photo-electric response consists essentially of two separate components, each with a negative potential defined above. At high light intensities these two components overlap one another and appear as a single peak.

There are two factors to be considered as regards the cause of the light-induced

response under investigation. The one is the different effects of the light in different regions of the spectrum and the other is the reaction supposed to take place in the leaf after the absorption of the light. There is a possibility of obtaining a two-peaked response if the leaf were equipped with two separate systems, each absorbing light of different wave lengths and reacting in a separate way, and with a different time of response. An alternative explanation would be to assume that there are two response systems with different reaction time, but with an identical (or common) action spectrum.

For the purpose of obtaining material basis to decide this point, a comparison was made between the effect of a monochromatic light and that of the white light in test-illumination. It was revealed that a monochromatic red light obtained by passing through an interference filter ($\lambda_{\max}$: 665 m μ , $T_{\max}$: 44.0%, $\Delta\lambda^{1/2}$: 14 m μ) gives rise to a photo-electric response with two peaks quite similar to that evoked by the white light at certain level of intensity. This fact indicates that the occurrence of two peaks is not attributable to the participation of two separate mechanisms with different action spectra, but supports the view that they are most probably caused by the difference in reaction time of the two mechanisms assumed to take place in the leaf after the absorption of the light. The physiological changes underlying the electrical response, however, still remain unelucidated.

It was revealed from the above-described results that the first negative phase of the response (i.e., rise in the potential curve) appears immediately after the illumination is begun. It was also noticed in some cases that a small "cusp" (i.e., drop in the potential curve) preceded the first negative phase of the response. The appearance of this "cusp" is most reasonably correlated with higher intensities of test-light, no such "cusp" being observed at light intensities lower than 2,000 lux (Cf., Figs. 1 and 2). It will also be noticed in these figures that the runs with a cusp at the beginning of light-period are marked by a transitory appearance of a small peak on turning off the illumination. The time, t , that elapsed before the second (main) peak in the potential curve appeared, on the other hand, was found to become more and more shortened as the light-intensity was increased, the two peaks finally being united into a single peak. The magnitude of the second peak is also a function of light-intensity, a high level being attained with stronger illumination. What immediately follows from these circumstances is that with higher light-intensities a potential curve is obtained which is characterized by the appearance of a small "cusp" at the commencement of the light-period followed by a main peak leading finally to a stationary level of potential (see Curve A, in Fig. 1).

The effect of light-intensity on the reaction time t (i.e., the time that elapses before the second, main peak) is illustrated in Fig. 5. The values in this figure are taken from the experimental results shown in Fig. 1. Similar experiments always give rise to approximately the same curve; a rectangular hyperbola. Tröndle⁴⁾ has investigated the effects of light on the permeability of protoplasm and found that the time that elapsed before the change in permeability occurred (i.e., reaction time) was a function of light intensity, and that the relation between the reaction time and the light intensity was given by a rectangular hyperbola. The reaction

Fig. 5. Reaction time (t) for the second peak of response as a function of intensity of illumination (circles; taken from Fig. 1 and the theoretical curve; calculated from Equation 1).

concerned. In this case, the reaction time, t , minus 0.6 min. may correspond to the presentation time (Präsentationszeit, Tröndle). Therefore, the above formula represents that the product of the light intensity and the presentation time is constant. It may be inferred from this fact that, in the photo-electric response under investigation, a certain process which presumably obeys the constant stimulus quantity-law is involved in a light receptor system in the leaf, and is accompanied by the potential change after the lapse of constant time (0.6 min.), independent of the light intensity.

The above-described results indicate that the intensities of the pre-illumination have a significant influence on the subsequent photo-electric responses and that the pre-illumination with intensity of radiation at constant energy have some different effects on the subsequent photo-electric responses caused by the same intensity of test-light. From these facts, it may be suggested that the photo-electric response in question is a really complicated reaction, in which certain secondary effects of some unstable intermediary substances formed as a result of pre-illumination may also be involved. Further investigations should be performed to make clear the actual mechanism of the events involved.

Summary

1. The changes in the photo-electric response in the leaves of *Phaseolus vulgaris* were investigated under different light-intensities and in relation to the pre-illumination.
2. The photo-electric responses of this material are essentially composed of two separate components in the negative phase as clearly shown in low light-intensities.
3. The response is increased by the pre-illumination of high intensity and is decreased by that of low intensity within certain limits.
4. The pre-illumination at a constant energy, but at varied intensities, of radiation does not result in an identical effect in response; i. e., the reciprocity-law does not hold in the pre-illumination.
5. A certain process which presumably obeys an extended form of the constant

time t (min.) in the present case can be calculated according to his equation⁴⁾

$$t = \frac{5 + (i-1) 0.6}{i}, \quad (1)$$

or

$$i(t-0.6) = 4.4 \quad (2)$$

where i is the intensity of the light in units of 1,000 lux.

This formula means that the product of the light intensity and the reaction time minus a constant (0.6 min. in the equation) is constant so far as the experimental conditions in Fig. 1 are

stimulus quantity-law is inferred to be involved in the reaction underlying the second negative phase of the response.

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References

- 1) Nishizaki, Y., Sci. Rep. RITU. D 9: 107 (1958). 2) ———, Kagaku (Science) 27: 464 (1957) (in Japanese). 3) Blinks, L. R., Jour. Gen. Physiol. 23: 495 (1939). 4) Tröndle, A., Jb. wiss. Bot. 48: 171 (1910).

摘 要

1. *Phaseolus vulgaris* の葉を用い、種々な強さの光を照射した場合に起こる光電反応の変化を、前照射の影響をも含めて研究した。
2. 光電反応は、強い光では 1 つの成分からなり、光が弱くなると反応は小さくなると同時に 2 つの成分に分離する。その移行過程から推して、強い光による反応は 2 つの成分が重なって現れたものであることが分る。
3. 強い前照射によって光電反応は大きくなり、逆に弱い前照射によって反応は小さくなる。
4. 前照射の光エネルギーが一定でも、次に起こる光電反応は一定にならず、前照射の強さ、照射時間の変化に従って増大あるいは減少がみられる。すなわち前照射に関しては、reciprocity-law が成立しない。
5. 光電反応の第 2 の成分の peak があらわれるまでの時間と光の強さとの関係は 1 つの直角双曲線で示され、この反応には刺激量法測に従がうようなある過程が含まれていることが推論される。