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EFFECTS OF LIGHT ON THE CULTURED RICE ROOTS[1]

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Abstract

Aseptic seminal roots of rice (*Oryza sativa* L.) excised from Taichung Native 1 and IR-8 were used as materials. Two root tips were cultured in a 125 ml Erlenmeyer flask containing 25 ml liquid R_2 type improved media. Cultures were maintained at 27-28°C for three weeks. Light treatments were conducted by changing the light sources, light intensities, duration of exposures, light and dark interchange during the culture period. The growth and morphological changes of the roots were investigated and compared with those of the dark grown ones. Light treatments have shown significant effects on the growth rate and morphology of roots. Root length of the light-treated ones was almost one half of dark grown ones after three weeks culture. The growth of the roots inhibited by the light at the beginning could be recovered by the subsequent dark condition, and the rapid growth during the dark culture could be inhibited by the subsequent light condition. Different light intensities from 25, 50, 75 to 100 ft-c, have no difference in the inhibition of cultured roots growth. Further observations on the effects of light qualities on roots growth under the red light and far-red light have shown that the growth of roots could be inhibited by red light, but not by far-red light. The results suggested strongly that the light inhibition on the growth of cultured rice roots might be controlled by the photochemical reactions such as P_r and P_{fr} reversible phytochrome system.

Introduction

Many kinds of excised plant roots have been aseptically cultured in artificially synthesized media since 1922, only those from dicotyledonous plants have been successful (Butcher and Street, 1964). Owing to some unknown factors, most of gramineae origins with a few exceptions (Ferguson, 1963; Street *et al.*, 1961) still can not get substantial progress. Rice plant is not an exception (Butcher and Street, 1964; Kawata *et al.*, 1967). After many experiments on the excised rice root culture since 1969, the senior author has

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obtained two types of improved culture media for Indica type rice root culture, namely R_1 and R_2 (Lai and Lee, 1971a, 1971b). The R_1 type culture medium has a typical composition as follows: modified White's inorganic salts plus 3% sucrose, 0.1 mg per litre of thiamin-HCl, pyridoxine-HCl and nicotinic acid, and 0.3% vitamin free casamino acids. The initial pH value was adjusted to 5.0. The medium has the activity in promoting the growth of excised rice roots, however the rapid growth rate declined quickly after two weeks from the inoculation. The R_2 type culture medium has a typical composition as follows: modified White's inorganic salts plus 5% sucrose, 0.1 mg per litre of thiamin-HCl, pyridoxine-HCl and nicotinic acid, but casamino acids were excluded completely from the medium. The initial pH value was adjusted to 4.0. Excised roots grew normally in this medium even after two weeks from inoculation. The above cultural experiments were mainly maintained at 27–28°C, and kept in dark condition during the culture period.

After the primary success in finding out the culture media for excised rice root culture, a series of factors which may influence the cultural results were re-examined using mainly R_2 type medium. The effect of illumination on the excised root culture was typically interesting one, which will be reported here.

Materials and Methods

The seminal root tips excised aseptically from rice cultivar (*Oryza sativa* L.) Taichung Native 1 and IR-8 were used as materials. Dehulled rice seeds were sterilized with 0.1% mercuric chloride solution for about 1 minute and then transferred to 10% calcium hypochloride solution for another two hours at 5°C. After the sterilization, excess calcium hypochloride was washed out with sterilized distilled water. The seeds were placed on 2.5% agar bed in petri dish, incubated for germination at 27–28°C in dark condition. After the germination two root tips, each about 8 mm in length, were excised and cultured in a 125 ml Erlenmeyer flask which contains 25 ml liquid culture medium of R_2 type. Most cultures were maintained at 27–28°C for about three weeks. The necessary light treatments were conducted by changing the light sources, light intensities, duration of exposures, or changing reciprocally light and dark conditions during the culture period. The different kinds of light intensity used were obtained by adjusting the distance between the light source and flask and estimated with SPI-1 type Toshiba's lux meter and YSI 65A type radio meter. The whole experiments were carried out in a artificially lit room of phytotron, Taiwan Agricultural Research Center. The temperature was kept at 27–28°C during the culture period. The growth rate and morphological appearance of the roots were investigated and compared with

those of the dark grown ones.

Results

The growth of cultured excised roots, which were exposed to 12 hours per day of 100 ft-c light intensity with the high pressure mercury plant lux lamp hung over the ceiling of lit room, was inhibited significantly as compared with that of the dark cultured ones. The root length was almost one half of the dark grown ones during three weeks culture (Fig. 1). The appearance of the roots responded to the light was quite alike between the roots of Taichung Native 1 and IR-8.

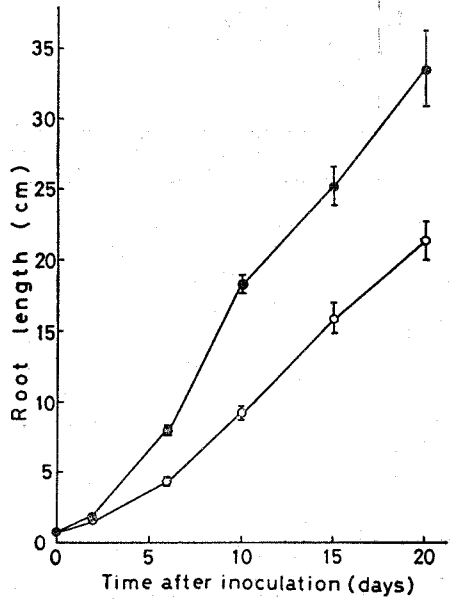

Fig. 1. Rice root growth during 3 weeks culture in 100 ft-c light intensity exposured 12 hours per day and in dark condition (Cultivar: Taichung Native 1).
●—● dark condition; ○—○ light condition.

Rice root cultures were conducted in light and dark conditions maintained 10 days at the begining. The light was conducted with 100 ft-c light intensity and kept for 24 hours continuous illumination. After then, half of light treated cultures were shifted to dark condition, and vice versa. The growth of the roots which were inhibited by the light at the begining, could be recovered by subsequent dark condition, and the rapid growth rate during the dark culture could be inhibited immediately by the subsequent light treatment

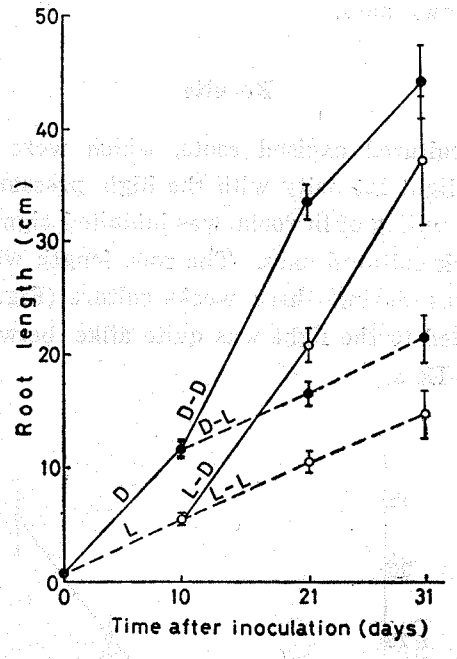

Fig. 2. The responses of cultured root to the dark and light conditions as well as reciprocal interchange of the conditions during culture period (Taichung Native 1). D-D: whole culture period kept in the dark; D-L: initial 10 days kept in the dark, then continuously exposured to 100 ft-c untill 31th day; L-L: whole culture period kept in light condition; L-D: initial 10 days kept in light condition, then kept entirely in dark.

(Fig. 2). Different light intensities, 25, 50, 75 and 100 ft-c respectively, have no difference in the inhibition of cultured root growth as compared with that of the completely dark grown one (Fig. 3).

The roots illuminated with the weak red light, instead of the above mentioned light source, by using white fluorescent lamp wrapped with two layers of red cellophane papers (about 6-7 ft-c on the surface of the flask) were also greatly inhibited as compared with the dark grown one.

Further studies on the effects of different light qualities on the growth of cultured roots were compared with that of dark condition. The light sources were obtained as follow:

red light—10 W white fluorescent lamp wrapped with two layers of cellophane papers.

white light—irradiated with 60 W white incandescent lamp.

far-red light—irradiated with far-red lamp directly.

The intensity of the different light sources were adjusted to 1 to 3 joules/m²/sec. near to the surface of flask with the aid of YSI 65A type radio meter.

The results show that the growth of cultured roots could be inhibited by red light and white light only, but not by far-red light at all (Fig. 4).

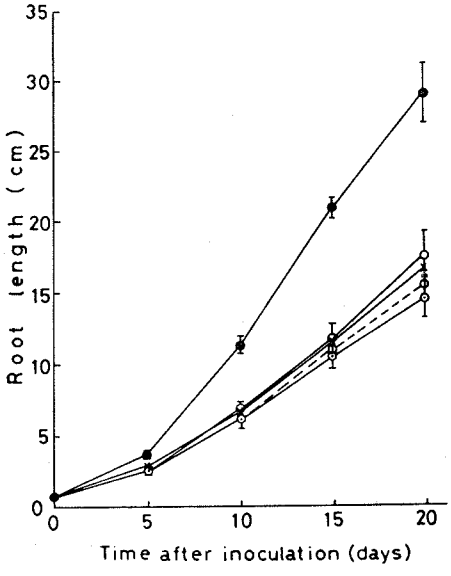

Fig. 3. The responses of root growth to different light intensities from 25 to 100 ft-c.
●——● dark; ×——× 25 ft-c; ○- - -○ 50 ft-c; ⊙——⊙ 75 ft-c; ○——○ 100 ft-c.

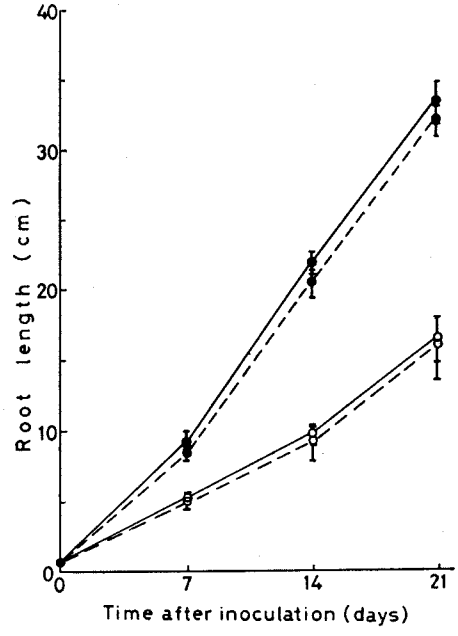

Fig. 4. The responses of cultured root growth to the different light sources during 21 day period.
●——● dark; ●- - -● far-red light for whole culture period; ○——○ white incandecent light for whole culture period; ○- - -○ red light for whole culture period.

The morphological differences of roots were also very significant between the light-treated ones and the dark-grown ones. The roots cultured under light conditions became thicker, zigzag instead of straight, and the elongated root hairs from the epidermis were differentiated significantly. In their cortical cells, very conspicuous starch grains were formed as compared with the dark grown ones (Fig. 5, 6 and Table 1, 2).

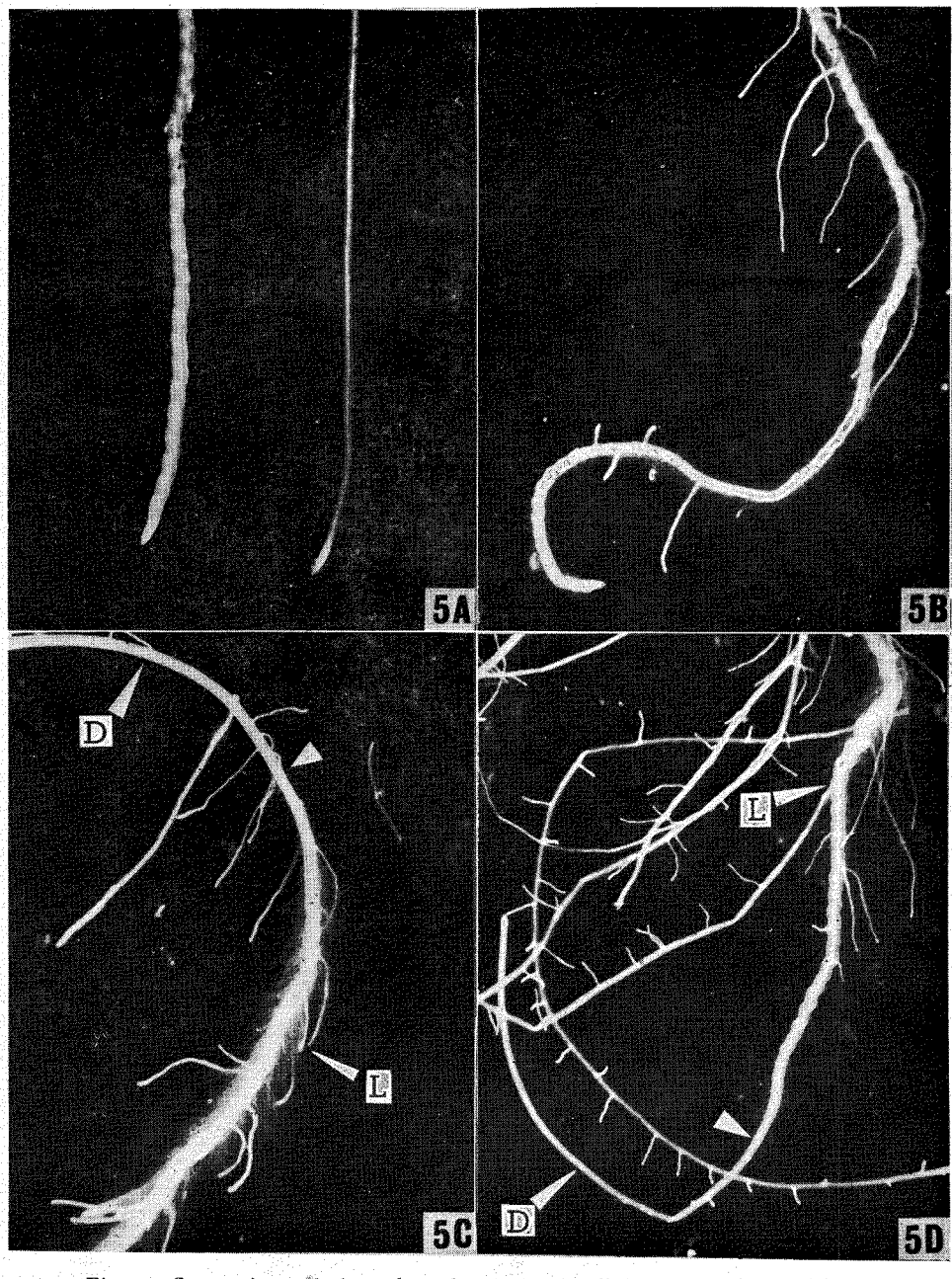

Fig. 5. Comparison of the cultured roots under light and dark conditions.

A: Outer morphology of root tips. left: cultured in the light; right: cultured in the dark. **B**: A root cultured in light condition became zigzag and differentiated long root hairs near the root apex. **C**: Parts of a root show dark grown (D) and light grown (L). "△" shows the inflection point of dark light interchange of the culture condition. **D**: Parts of a root show light grown (L) and dark grown (D). "△" shows the inflection point of light dark interchange of the culture condition.

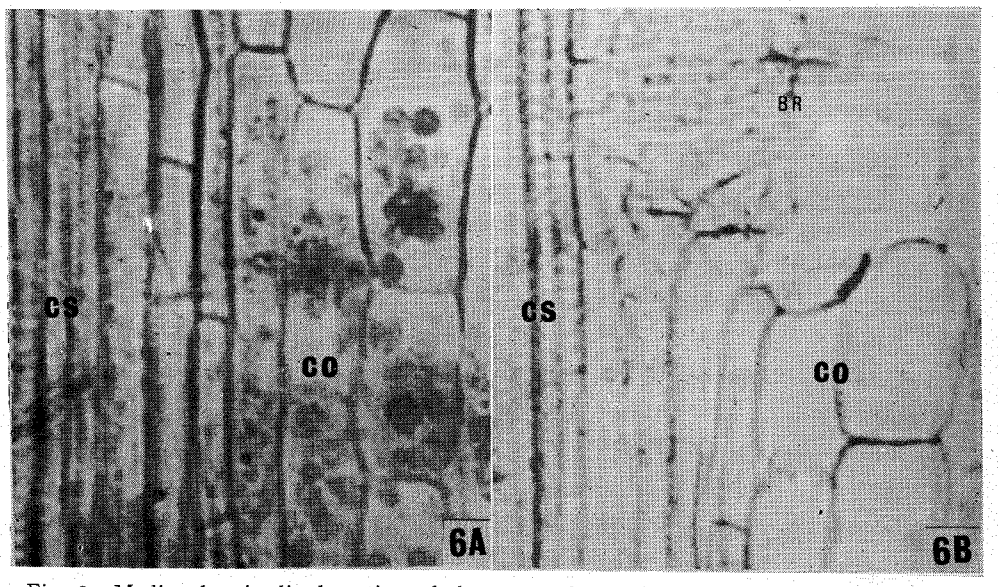

Fig. 6. Median longitudinal section of the root cultured in the light and in the dark.

A: In the light. CS: central cylinder; CO: cortical parenchyma cells with many starch grains. **B**: In the dark. CS: central cylinder; CO: cortical parenchyma cells; BR: branching root.

Table 1. *The thickness of root and the length of root hair differentiated under dark and light culture conditions*

Culture condition	Root diameter	Root hair length
Dark	320±16 μ	undifferentiated
Light	389±15 μ	765±51 μ

Table 2. *The accumulation of starch grains in the different tissues of cultured root*

Culture condition	epidermis	cortex	central cylinder
Dark	—	—	—
Light	—	+++	—

Discussion

Root is a subterranean organ of the plant body. Some investigators have described direct effects of illumination on the root growth. Among them, Segelitz (1938) has reported that isolated corn roots grown in culture in the dark extended more rapidly than the roots exposed to white light, conceived to be an effect attributed to the production of auxin by roots in the light. Robbins and Maneval (1924) reported that the growth of excised corn roots was favorably influenced by light. However, White (1937) has stated that light has no observable effect on the growth of tomato roots in a sterile nutrient medium. Robbins (1940) reported that a peculiar periodic formation of root hairs by isolated roots of Datura which were grown in a sterile culture. Root hair formation occured during day light period and the rate of root elongation was reduced thereafter.

The responses of root growth to the light in different plant materials can be summarized in three categories: namely inhibitory, enhancing and no effect at all. Apparently, the growth behavior of rice root belongs to the first one. Among three elements of light, it is a prime importance to be considered that the light quality extended most obvious effect on the root growth. As the duration of illumination prolonged to 24 hours, the growth rate of root length was greatly reduced, as compared with that illuminated 12 hours per day. And there were not different at all in growth inhibition among light intensities treated within the range of 25 to 100 ft-c. The results obtained from the treatments of red and far red light suggested that some red and far-red reversible physiological entities were in operation on the growth of rice root.

Ohno and Fujiwara (1967) have reported that rice root growth in water was not completely affected by a brief red irradiation but was inhibited by continuous red illumination. The spectrophotometrical analysis of phytochrome was carried out, however, the intimate correlation between the phytochrome in vivo and physiological growth inhibition was not found.

Recently Pjon and Furuya (1968) have demonstrated that the growth of rice seed coleoptile belongs to a typical phytochrome controlled reaction. Spectrophotometrical analysis has proved the good correlation between the in vivo phytochrome and the elongation of coleoptile.

The results obtained from the above experiments, strongly suggested the light inhibition of the cultured rice roots might belong to a photochemical reactions of the root metabolism, such as P_r and P_{fr} reversible phytochrome system.

Moreover, the morphological appearance induced by light treatment is

worth to discuss in the view of photomorphogenesis. As the experiment results have shown, light-treated roots have differentiated many long root hairs, and in their cortical layer cells conspicuous starch grains were found. The root, thicker in girth and zigzag with many long root hairs at one hand, and slender, straight, root hairless in another, could be obtained by changing the light and dark conditions during cultured period. These facts suggested some reversible enzymic systems of starch formation in the cortical layer and RNA synthesis in the root epidermal cell may be influenced by light (Kawata and Ishihara, 1961). Thus it is worth of consideration that the physiological and morphogenetical aspects of the roots under light and dark conditions deserve further study.

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光對水稻分離根生育之影響

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爲研究稻根生理生態之一環，利用在人工培養基中能做正常生長之分離根觀察稻根對光之生育反應，茲把該結果摘要於後：即使用水稻品種臺中在來1號和 IR-8 號無菌分離根爲材料，把分離根接種於盛有25毫升 R_2 型改良培養基之 125 毫升三角瓶內培養。培養溫度保持 27-28°C。培養期間以不同光質光源，光强，光照時數或明暗交互處理培養根並觀察其生長及根內、外部形態和始終置於暗中培養的分離根比較。結果，以光强 100 ft-c 照射12小時/日的分離根，根長的伸長受光之抑制而明顯地減少。初期保持在暗中培養的分離根，其中一部於培養中途移出並以光照處理者，則原在暗中伸長正常的根即時受光之照射影響，其伸長受明顯之抑制。反之，在培養初期以光照處理，根長伸長明顯受抑制之分離根，經移入暗中後即迅速恢復正常之伸長生長。處理光强在 25 ft-c 至 100 ft-c 的範圍，對分離根之伸長抑制程度而言，無明顯之差異。以光强僅爲 6-7 ft-c 之弱紅光代替白色光照射分離根，分離根之伸長亦受到顯著抑制。利用不同光質光源（强度爲 1-3 joules/m^2/sec.）處理分離根，結果顯示白色光，紅光均能顯著地抑制分離根之伸長，但以紅外光處理則和暗中生長者類似，不表現任何抑制作用。再以分離根之內、外部形態比較觀察之結果，受白光、紅光處理的分離根，根徑較暗中生長者爲粗大，形狀成爲扭曲狀，並自根表皮分化數目極多，長度較長的根毛，且其皮層組織存有極多明顯的澱粉粒。反之，在暗中生長的分離根，根徑較細，成直線狀伸長，根表幾無根毛之分化，又根內皮層組織亦缺少明顯之澱粉粒。

由以上觀察結果，可得一推論即光對水稻分離根生育之影響可能爲屬於一種 P_r（紅色光）$\rightleftarrows P_{fr}$（紅外光）系之光反應，亦即紅光對它的生育有極明顯之抑制及光形態形成作用，而紅外光則不然。