STUDIES ON THE POLYACRYLAMIDE-GEL ELECTROPHORESIS OF RICE ISOZYMES[1]

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(Received Feb. 6, 1969)

Introduction

Recent studies in comparative analysis of protein and isozymes (Markert and Møller, 1959) have been the subject of many investigations. The disc electrophoresis has been proved as a remarkable new technique to achieve these aims (Reisfeld *et al.*, 1962). The proper histochemical staining and zone electrophoresis have made it possible to study electrophoretic variations in various isozymes. Many of these works have been done in man (Karp and Sutton, 1967), fish, silkworm (Yoshitake and Akiyama, 1964), chicken, Drosophila (MacIntyre, 1966), corn (Schwartz and Endo, 1966, 1966), rice (Chu, 1967), with starch (Smithies, 1955) or polyacrylamide gel.

The irraditation effects on enzyme systems in higher plants have been reported by the analysis of specific and total enzyme activities (Schwartz, 1960, Haskins and Chapman, 1956, Gunkel and Sparrow, 1961). The analyses were based on the specificity of enzyme reactions coupling with zymogens, and these were only a average estimate of particular isozymes. The effects of irradiation to the individual isozyme can also be detected by comparison of the intensities of isozyme bands in zymograms after electrophoretic separation of the multiple forms of the enzyme, but the results are difficult to quantify.

Taichung No. 65 is a Japonica type of rice that is cultivated in Taiwan. In 1962, a mutant line was selected after X-rays and γ-rays irradiation treatment. Since Taichung No. 65 mutant is very similar to Taichung No. 65 in morphological and physiological characters except earlier maturity stage of about ten days, there must be some internal differences which exist between these two varieties.

The present paper reports some enzyme variations between these two varieties. The enzymes analysed in this experiment were arylesterases, aldehyde dehydrogenases, alcohol dehydrogenases and xanthine oxidase (Dixon and Webb, 1964).

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Materials and Methods

Dry seeds of Taichung No. 65 and its mutant were soaked in 0.1% $HgCl_2$ solution for 5 minutes, and then washed. After 24 hours of incubation at 30°C in the dark, the seeds were cultivated under uniform conditions in water culture solution. 14 days after germination the seedlings were prepared for sampling.

Preparation of extracts

20 g of seedlings in fresh weight were minced into a motor with an equal volumn of quartz and 100 ml of 0.1 M tris-borate-EDTA buffer containing 5% sucrose, pH 8.9. The mixture were ground to homogeneous state. The macerate was squeezed through two layers of cheese-cloth. The dark-green liquid was centrifuged with low speed to remove the cellular debris and quartz. The turbid supernatant was sonificated for 5 minutes, then spun at 20,000×g for 20 minutes. The clear supernatant was saturated with ammonium sulfate to 30%, standing for 15 minutes, then centrufuged at 10,000×g for 30 minutes. The pellet was discarded and the supernatant was saturated with ammonium sulfate again. The 30–60% and 60–100% ammonium sulfate precipitation fraction were prepared respectively as described above, and dialyzed overnight against 1×10^{-3}M tris-borate-EDTA buffer, pH 8.9, so as to remove ammonium sulfate. The dialysate was satuated with 0.1 M tris-borate-EDTA buffer containing 10% sucrose, and sucrose concentration was adjusted to get a final sucrose concentration to 2.5%. The protein extract was then stored at -27°C. All the operations were carried out in a cold room at 4–6°C.

Preparation of gel column

5% polyacrylamide gel was made according to Nerenberg (1966) with some modifications. The acrylamide gel was prepared by using 0.1 M tris-borate-EDTA buffer, pH 8.9, instead of glycine tris buffer. Glass tube (6–mm internal diameter) cut to lengths to about 6.5 cm. A solution of 30 ml of 0.1 M tris-borate-EDTA containing 5% acrylamide was taken, then 0.5 ml of 10% ammonium persulfate solution was added, mixed well and injected into the glass tube. After 5–10 minutes, the gel was polymerized. Gels with the same length glass tubes were selected for electrophoresis.

Electrophoresis

The acrylamide gel vertical electrophoresis was performed by using 0.1 M tris-borate-EDTA buffer system, pH 8.9. 50 μl of the sample was applied to the surface of a gel by means of a capillary. The remaining space in each glass tube was filled with tray buffer. Electrophoresis was carried out by applying a current of 5 m. a. per tube for 45 minutes. When electrophoresis was completed, the gels were removed from the glass tube by rimming gels with a

22-gauge syringe needle. The gels were fixed and stained with diffreent procedures so as to detect different isozymes.

Arylesterases were stained by the method as follows: The gels removed from columns were incubated in 0.5 M boric acid in cold room for 1 to 1.5 hours. Then the staining was performed in 50 ml of 0.1 M Na-phosphate buffer, pH 6.5, containing 0.5 mg/ml of Fast red salt TRN and 4 mM of α-naphthylacetate or β-naphthylacetate (dissolved in 2 ml of 50% acetone previously). After 1 to 2 hours incubation at room temperature or overnight in cold room, the staining was completed.

Aldehyde dehydrogenases were stained in 50 ml tris-HCl buffer, pH 8.5, containing 0.25 mM of NAD, 0.15 mM of p-nitro blue tetrazolium chloride (Nitro BT) and 4 ml of acetaldehyde. Incubation was carried out for one hour in room temperature, then 2 mg of phenazine methosulfate (PMS) was added. After 1 to 2 hours incubation in the dark, gels were rinsed and stored in 7% acetic acid to prevent further staining.

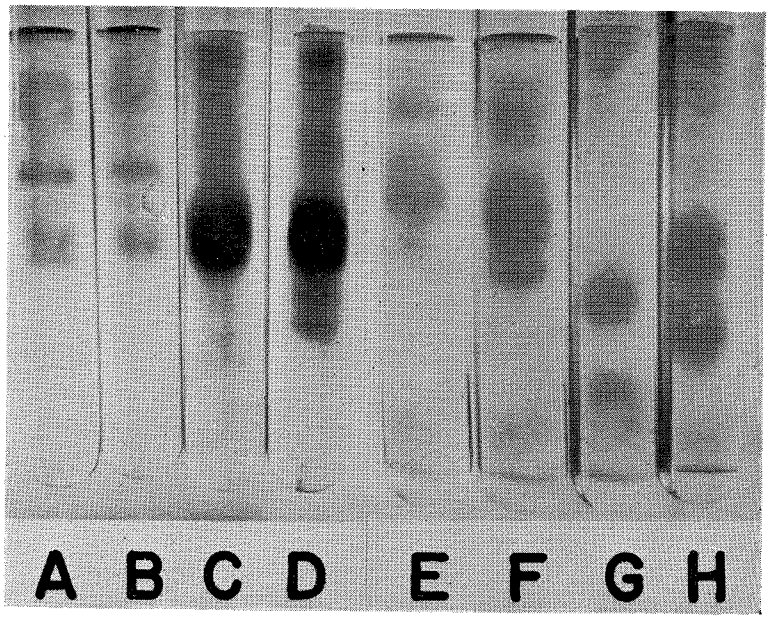
Alcohol dehydrogenases were stained in 50 ml of 0.1 M tris-HCl buffer, pH 8.5, containing 0.3 mM of NAD, 0.12 mM of Nitro BT and 0.4 ml of iso-propanol. Incubation was carried out for one hour at room temperature, then 1 mg of PMS was added. The same procedures were performed as described above.

Xanthine oxidase were stained in 50 ml of 0.1 M tris-HCl buffer, pH 7.5, containing 0.25 mM of NAD, 0.3 mM of Nitro BT, 2 mM of KCN and 4 ml of 0.05 M hypoxanthine (dissolved in 0.1 N HCl). The same procedures were performed as described above.

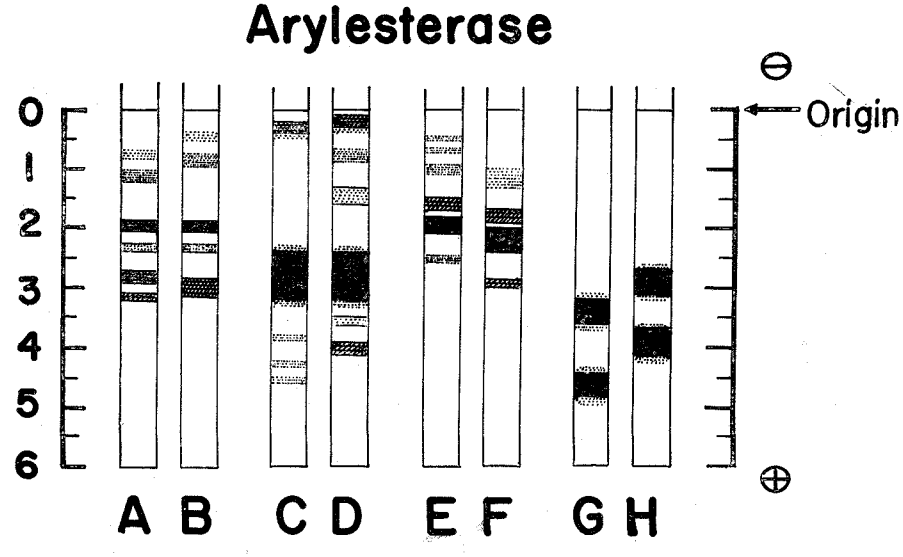
Results and Discussion

The enzyme variation in the young seedlings between these two varieties were shown in Figure 1 to 4. Each of these enzymes had been replicated at least five times under similar conditions. Since some zymograms were too faint to have the taken photos, so all the results are represented by diagramatic drawings. Different intensities of its zymograms have been indicated by different drawings. The results of these experiments are discussed below.

Arylesterases: The zymograms had shown some extent overlapping by adding both α-naphthylacetate and β-naphthylacetate. In order to be more accurate, zymograms were detected separately by different substrated (Figure 1, 2). In 30–60% ammonium sulfate fraction, a considerable decrease in the number of isozyme bands was observed in Taichung No. 65 mutant both in alpha and beta naphthylacetate as substrate. On the other hand, only a slight difference could be detected in 60–100% ammonium sulfate fraction. However, in both fractions, the main difference between these two varieties was the change of electrophoretic mobility.



igure 1. Zymograms of young seedling arylesterases isozymes (left to right): (A) and (E)=30-60% ammonium sulfate precipitation fraction (A. S.) of Taichung No. 65; (B) and (F)=30-60% A. S. fraction of Taichung No. 65 mutant; (C) and (G)=60-100% A. S. fraction of Taichung No. 65; (D) and (H)=60-100% A. S. fraction of Taichung No. 65 mutant. From (A) to (D) and (E) to (H), the substrates for enzyme reactions were α-naphthylacetate and β-naphthylacetate, respectively.



igure 2. Diagramatic drawings of polyacrylamide-gel electrophoresis pattern of young seedling arylesterases. The alphabatic notes are the same as described in Figure 1.

Aldehyde dehydrogenases and Alcohol dehydrogenases: These enzymes were located in 30–60% ammonium sulfate fraction only. In the zymograms (Figure 3), three isozymes bands with slightly different electrophoretic mobility were detected in aldehyde dehydrogenases, where in alcohol dehydrogenases, only one band was observed in both varieties with slight difference in migration rate. (Figure 3)

Xanthine oxidases: The zymograms had shown the location of these enzymes (Figure 4). In the upper part of the gel, two isozyme bands were observed in both varieties with a similar migration rate. In the lower part of the gel, an increase of isozyme band was found in Taichung No. 65 mutant.

In conclusion, the effects of irradiation induced enzyme changes were: decrease in isozyme bands in 30–60% ammonium sulfate fraction arylesterases (Figure 1, 2), increase in isozyme bands of 30–60% ammonium sulfate fraction xanthine oxidases (Figure 4), and change the electrophoretic mobility of almost the whole enzymes tested.

The possible explanation of changes in isozymes may be explained as: (1) The decrease of isozyme bands is probably caused by the deletion of segamental DNA, whereas the increase is caused by the disturbance of normal controlling system of repression. (2) The changes in electrophoretic mobility would

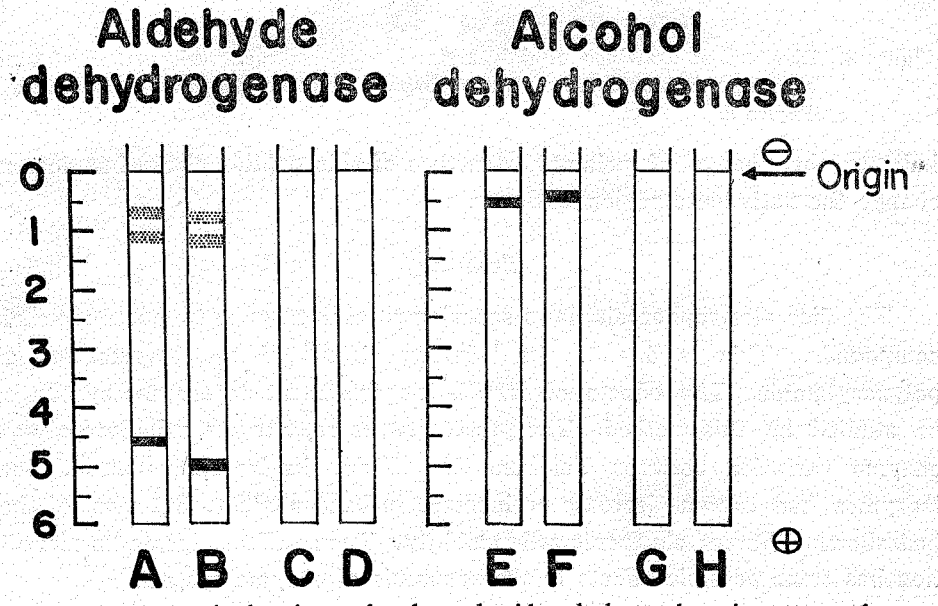


Figure 3. Diagramatic drawings of polyacrylamide-gel electrophoresis pattern of young seedling aldehyde dehydrogenases and alcohol dehydrogenases. (left to right): (A) and (E)=30-60% A.S. fraction of Taichung No. 65; (B) and (F)=30-60% A.S. fraction of Taichung No. 65 mutant; (C) and (G)=60-100 A.S. fraction of Taichung No. 65; (D) and (H)=60-100% A.S. fraction of Taichung No. 65 mutant.

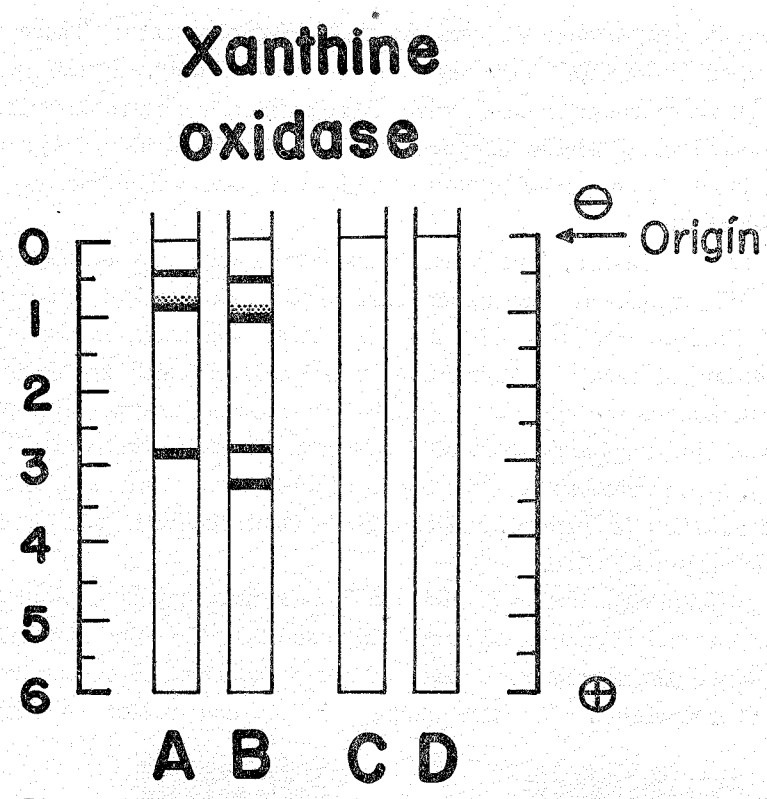


Figure 4. Diagramatic drawings of polyacrylamide-gel electrophoresis pattern of young seedling xanthine oxidase. The alphabatic notes are the same as described in Figure 3.

indicate the change of primary structure of this peptide chain, but dose not change the active-site region however.

Summary

The disc electrophoresis has a superior resolving power to macro-molecular compounds. This is due to the "sieving effect" of the polymerized gel, polyacrylamide. The effects of irradiation treatment to enzyme system can be studied by this method. The present paper reports the existence of some enzyme variation between Taichung No. 65 and its irradiate mutant. Some isozymes had shown decrease or increase in band number, but most of them had shown different electrophoretic mobility. The results suggests that irradiation has some possible efforts to the synthesis of enzyme system.

Polyacrylamide 膠凝體電泳法對水稻體內 Isozyme 的研究

馬 馭 李 先 聞

Disc electrophoresis 對高分子量物質具有優異的解析能力，此爲 polyacrylamide 膠凝體的篩濾效果。放射線對酵素系統引起的改變可用此法研討，本實驗以臺中65號及其放射線處理後得到的突變系爲材料進行此方面的研究，發現一部份酵素的 isozyme 有增減的現象，但大多數的差異爲各相對位置的 isozyme 有不同的電泳速度，此結果可以說明放射線對酵素生成之機構有改變的作用。

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