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Separation of Barley Seed Proteins by Disc Electrophoresis

Jong Yol Choi

Kangwon University

Disc electrophoresis에 依한 大麥種子蛋白質의 分離

崔 鍾 烈

江原大學

Introduction

Since the use of polyacrylamide gels as a supporting medium for electrophoretic separations was developed by Raymond et al(18, 19) and Ornstein and Davis (2, 16, 17), it has sprung into widespread use because of its powerful resolution capabilities in the analysis of and separation of complex mixtures of proteins, enzymes, hormones, peptides, nucleic acids and other biological materials.

Chang et al (1) compared paper, starch and disc electrophoresis, and detected 6-8 bands on paper, 18 on starch and 25 on disc electrophoregrams of soluble protein preparations made from a mutant strain of Neurospora crassa. Steward and Barber(21) applied disc electrophoresis to the separation of soluble proteins of Neurospora, tulip and pea. They reported that the method of protein extration as well as of gel polymerization had a bearing on the definition of bands ultimately obtained. Eldgidge et al(3) and Larsen(11) in soybean, and Sastry and Virupaksha(20) in sorghum separated seed proteins by polyacrylamide and disc eletrophoresis. Steward et al(22), in disc electrophoresis of sueccessive segments of pea seedlings, and El-Schafie and Davis(4), in successive leaves of onion bulb, showed that meristematic tissues contained more proteins, quantitatively and qualitatively, than mature tissues. Johnson and Hall(8) separated crude seed protein extracts of wheat and wheat relations by electrophoresis on polyacrylamide gels and suggested that electrophoretic separation of proteins may provide new insight into some problems of evolution and systematics. Johnson et al(9) obtained protein spectra from 4n and 6n species of Triticum by electrophoresis of seed extracts on polyacrylamide gel. Comparison of protein mixture spectra showed that homologies among the spectra were consistent with evidence from conventional methods regarding genome relationships among Triticum polyploids. Johnson (10) confirmed the genome donor of Aegilops cylindrica from the electrophoretic patterns of protein mixture of Ae. caudata and Ae. squarrosa. Thomas and Jones(24), by comparison of disc electrophoretic separation of the diploid species and cultivated hexaploid oats, confirmed the tentative conclusion for genome analysis that Avena ventricosa is the donor of the C genome.

Enary and Mikola(5) fractionated barley albumin by chromatography on DEAE-cellulose and distinguished 16 different components. Nummi and Enary(15) separated the water soluble proteins of barley grain into two fractions by gel filtration on Sephadex G25, G50 and G75. Enary et al(6, 7) obtained 16 bands of significant intensity on electro-phoregram of soluble barley proteins separated by starch gel electrophoresis. Nilson and Hermelin(14) demonstrated the variation in the isozyme patterns of barley varieties by the zymogram technique combined with starch gel electrophoresis. The basic patterns of mobility were shown to be species specific with some intraspecific variation. Mikola (13) studied, by disc electrophoresis, the changes wh-

ich occur in what he terms the basic proteins of barley during germination and found very few qualitative changes.

In the present paper, the results obtained from the disc electrophoretic separation of barley seed proteins have been reported.

1. Mateials and Methods

Seed samples of fifteen barley varieties representing winter 6-rowed, spring 6-rowed and spring 2-rowed group were used(Table1).

Table 1. Fifteen barley varieties used for seed protein separation.

Group	Variety	Collected from
Winter 6-rowed	1. Yongwol Yukkak	Korea
	2. ChangmanYukkak	Korea
	3. Tongmaek	Korea
	4. Chechon Ilho	Korea
	5. Kyong Ilho	Korea
Spring 6-rowed	6. Hiland	U.S.A.
	7. Traill	U.S.A.
	8. Trebi	U.S.A.
	9. Plaines	U.S.A.
	10. Wanju	Korea
Spring 2-rowed	11. Ingrid	Sweden
	12. Sv. Bonus	Sweden
	13. Ert. 23	Sweden
	14. Sv. Forma	Sweden
	15. Ert. C47	Sweden

Protein extraction. Seeds were finely ground using motar and pestle, and the powder was passed through 60-mesh sieve. One gram of the sieved powder was mixed with 6ml of tris-glycine buffer. After an overnight extraction at 4°C, the gruel was centrifuged at 2000×g for 30 minutes, the pellet discarded and the supernatant used for separation.

Electrophoresis. Electrophoresis was performed using the procedures described by Ornstein and Davis(2, 16, 17).

The Recording of the results. The gels were analysed densitometrically. Photographs were taken against a diffuse white background, illuminated from the rear. Prints made from negatives were enlarged to the length which correspond exactly to that of the densitometric traces. A diagrammatic interpretation was made for each gel, as some of fine and faint bands in the gels did not show well on either the densitometric traces or on photographs.

2. Results and Discussion

The banding patterns were highly reproducible following standardization of the protein extraction and electrophoretic procedures. Differences among replicates in each variety sample were negligible.

Densitometric traces of the gels are presented in Fig. 1 and Fig. 2 with photographs in juxtaposition to make comparison possible. Diagrammatic representation is also presented in Fig.3. The numbers attached to each variety in Fig. 3 correspond to the numbers prefixed to each variety in Table 1. In each gel 18 to 21 bands were visible. The electrophoregrams were tentatively divided into 6 major zones and numbers were allocated to each band for ease of identification.

A zone. All varieties showed three faint bands except Trebi. Trebi was the only variety that showed A5 band, Al band, very close to the front, could be seen only in Ert. C47. A4 band, however, could not be detected in this variety. Resolution in this zone either by visual or by densitometric was, however, so low that critical identification was difficult.

B zone. Three bands were visible in all varieties. B1 was common to all groups. Its intensity, however, was variable not only among groups but also within group. Four varieties in spring 2-rowed group showed dense B1 band (12, 13, 14 and 15 in Fig. 3). One variety in spring 2-rowed, two varieties in spring 6-rowed and four varieties in winter 6-rowed group showed less dense B1 band(2, 3, 4, 5, 8, 9 and 11). Three varieties in spring 6-rowed and one variety in winter 6-rowed group showed weak B1 band(1, 6, 7 and 10). B2 band was also common to all groups. Four varieties in spring 6-rowed group and one variety in spring 2-rowed showed dense B2 band(7, 8, 9, 10 and 11), and four varieties in spring 2-rowed and one variety in spring 6-rowed group showed less dense B2 band(6, 12, 13, 14 and 15). All varieties in winter 6-rowed group very faint B2 band. B3 band was detected only in spring barley and B4 only in

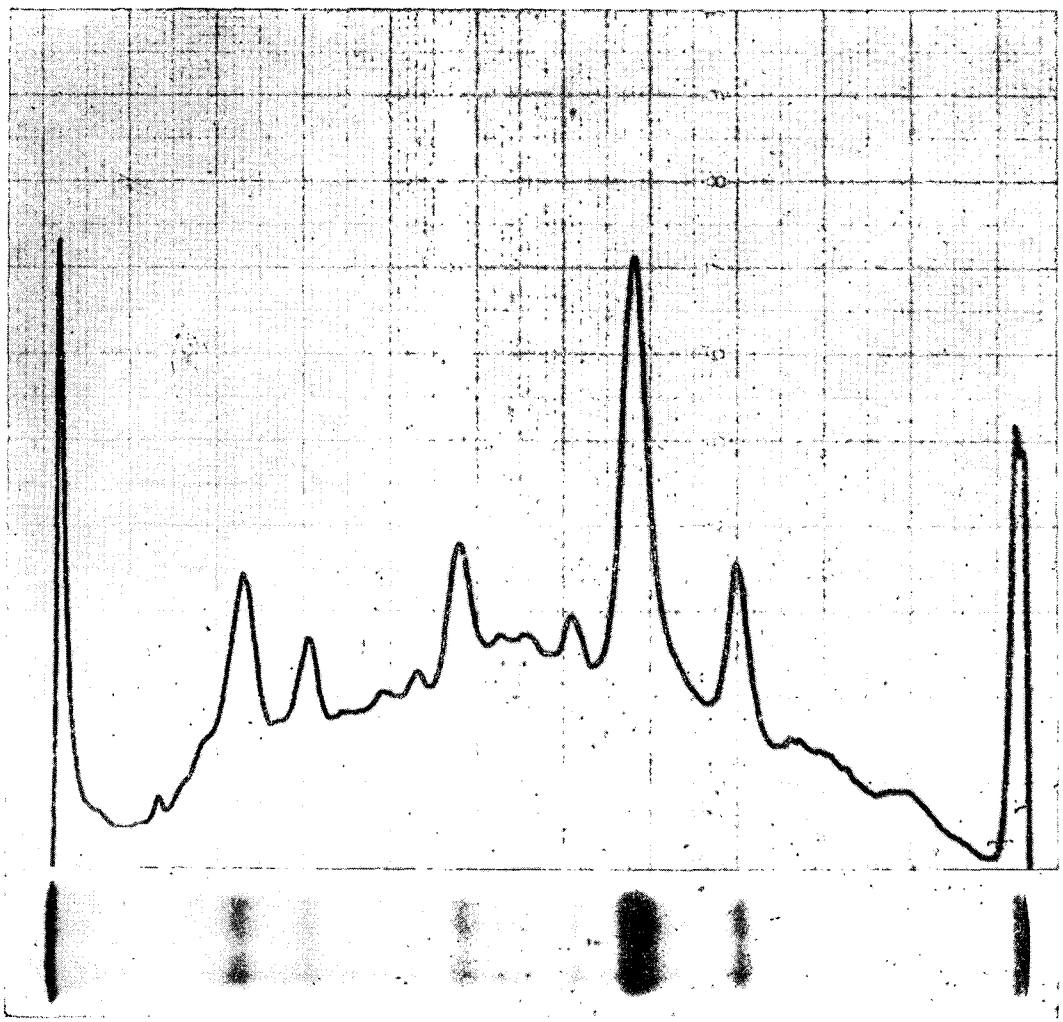

Fig 1. Electrophoregram of barley seed protein and densitometric trace (Changmang Yukkak).

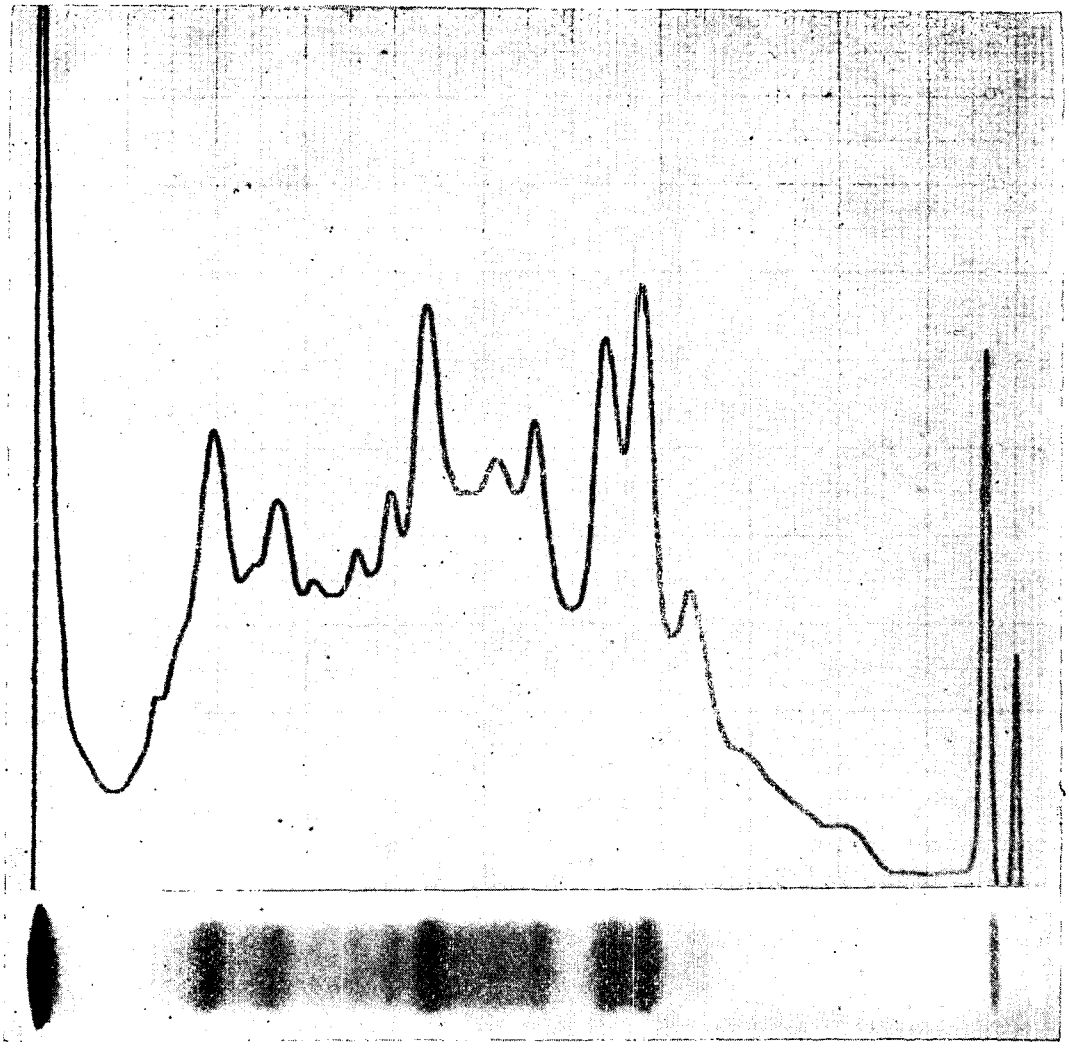

Fig 2. Electrophoregram of barley seed protein and densitometric tacer trace (Wanju).

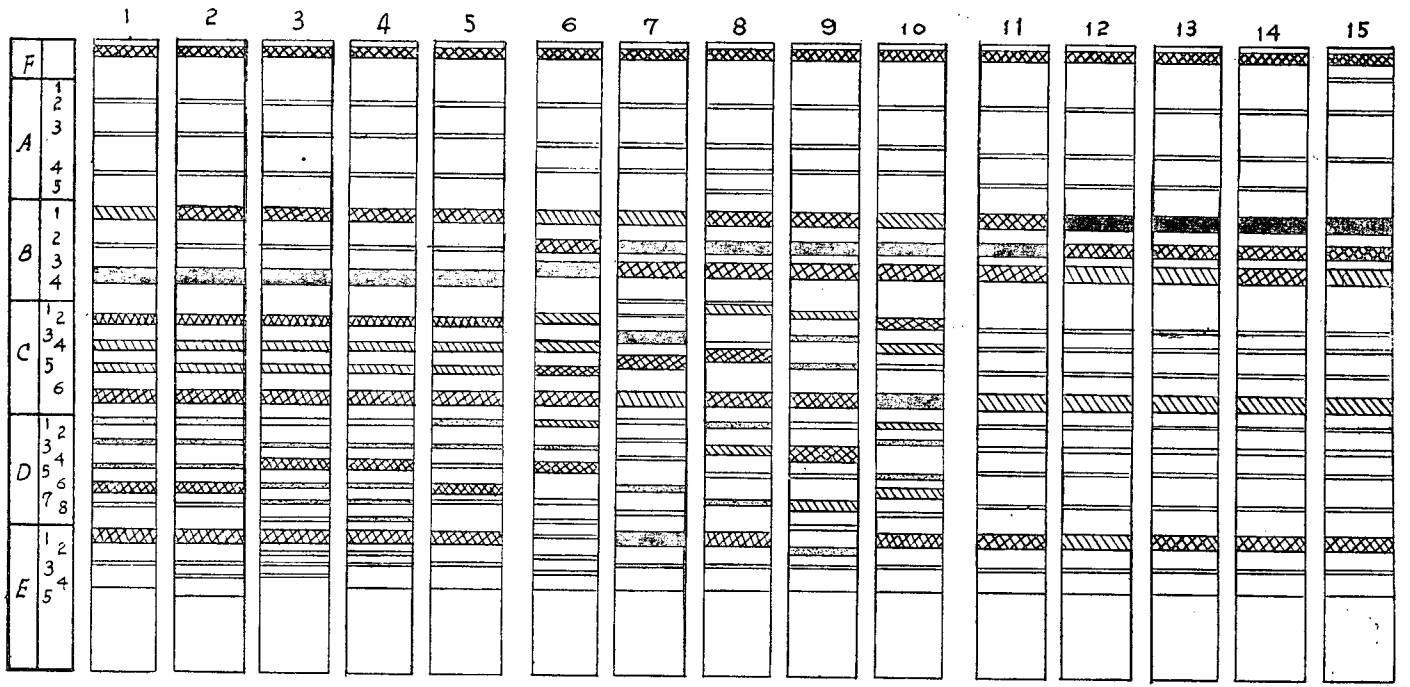

Fig 3. Diagrammatic representation of barley seed protein electrophoregrams (See Table 1 for identification of variety).

winter barley.

C zone. C6 band was common to all groups. Varietal differences in intensity of C6 band were visible only in spring 6-rowed group. Besides C6, winter 6-rowed had three weak and spring 2-rowed had three faint bands in common. The overall pattern in this zone was quite diversified in spring 6-rowed group.

D zone. Spring 2-rowed group showed four faint bands in common in this zone. Two types of banding pattern, however, could be noticed in winter 6-rowed group. Three varieties in this group showed, four faint bands and one less dense band in common(1, 2 and 5). Two varieties showed five faint bands and one less dense band in common(3 and 4). The banding pattern of this zone in spring 6-rowed group was multifarious.

E zone. E1 band was common to all varieties except Plaines(9). Plains showed E2 band instead of E1. E2 was very faint and slightly slower than E1 in mobility. Two faint bands were visible in all varieties of spring 2-rowed group(E3 and E4). Two varieties in winter 6-rowed group(1 and 5) and four varieties in spring 6-rowed(7, 8, 9 and 10) showed two faint bands. And three faint bands with different mobilities could be detected in three varieties of winter 6-rowed (2, 3 and 4) and in one variety of spring 6-rowed group(6).

The electrophoretic spectra disclosed by disc electrophoresis are species specific showing that the spectra are the reflection of hereditary constuents of a organism in one way or another. The bending patterns of barley seed proteins of fifteen varieties are different not only from those of other crops but also each other. However, it can be seen that closely related varieties show identical or nearly identical patterns. The possibility is still open, however, that the bands which showed the same mobility may contain different proteins and that technique may not be sufficiently sensitive to resolve them. To resolve such proteins, it would be necessary to elude them from the gel and subject them again to electrophoresis on another gel of different pore-size or pH. The present separation showed up to 21 components in soluble proteins of barley seed. Modern theory of molecular genetics, however, suggests that more complicated patterns could be expected. It is highly probable that more sensitive technique, if any, might disclose more diversified patterns to reveal finer differences which may exist among closely related genotypes.

3. Summary

Fifteen samples of barley seed proteins representing winter 6-rowed, spring 6-rowed and spring 2-rowed group were separated by disc electrophoresis.

Photographic, densitometric and diagrammatic representation were presented to make comparison possible. Eighteen to twenty one bands were detected in each gel. Electrophoretic spectrum of each variety was highly variety specific showing that the spectrum is the reflection of hereditary constituents of a organism. Related variety showed identical or nearly identical spectrum.

All winter 6-rowed barley showed very faint B2 band, while spring 6-rowed and spring 2-rowed barley showed dense or less dense B2 band. All spring barley showed B3 band, while all winter barley showed B4 band.

摘 要

春播二條, 春播六條 及 秋播六條大麥의 各 : 5品種 計 15品種의 種子蛋白質을 disc electrophoresis에 依하여 分離하고, 그 結果를 寫眞, densitometric tracing 及 模式圖로 表示했다.

品種에 따라 18~21 bands를 識別할 수 있었다. 品種은 各各 固有의 pattern을 보이고 있어, electrophoretic pattern이 各品種의 遺傳的 差異를 反映하는 것이라고 생각된다.

秋播大麥은 全部 稀薄한 B2 band를 갖이고 있고 春播大麥는 濃厚한 B2 band를 갖이고 있었으며, 또 B2 band는 春播大麥에서, B3 band는 秋播大麥에서만 볼 수 있어, 春播大麥과 秋播大麥를 electrophoretic pattern에 依하여 區別할 수 있었다.

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