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Two-Dimensional Electrophoretic Analysis of Rice Seed Proteins

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쌀 종자 단백질의 2차원 전기영동적 분석

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초 록

다수확계(통일벼) 및 일반계 각각 3가지 품종의 각 출수후 6시기의 쌀종자에서 2% sodium dodecyl sulfate/5% β-mercaptoethanol로 단백질을 추출하여 1차원에서 등전점에 따라 분리한 후 2차원에서 분자량에 따라 분리하는 2차원 전기영동으로 각각의 단백질 지도를 작성하였다. 종합단백질 지도를 작성하였고 pH 5.2~8.3, 분자량 20,000~100,000의 범위에서 300개 이상의 spots이 관찰되었다. 품종 및 출수시기에 따라 단백질 지도가 많은 차이를 보였다.

Introduction

The complex of proteins present in the seeds of various rice cultivars (*Oryza sativa L.*) has been proven difficult to resolve analytically, to interrelate functionally and genetically, and to name in a useful manner. The core of analytical problem seems to be that of identifying families of related genes and their protein products.

High-resolution two-dimensional (2-D) electrophoresis, with isoelectrofocusing in urea under either equilibrium or nonequlibrium conditions in the first dimension and electrophoresis in the presence of sodium dodecyl sulfate in the second is capable of resolving several thousands of proteins in one analysis[1~4], and has been previously applied to the anyalysis of rice glutelins[5].

Related techniques such as sodium dodecyl sulfate polyacrylamide gel electrophoresis(SDS-PAGE)[6~10] and electrofocusing[8,9,11] have been used to analyze rice albumins and globulins[6,8,9], albumins and prolamins[11], and glutelins[7,10].

The objective of this work was to construct a composite map of rice proteins by analyzing different rice varieties at various stages of development and to compare them, which would help identification of rice proteins and their relation to functions.

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Materials and Methods

Rice samples

Six rice cultivers-three Japonica types(Sobaek, Sangpoong and Choochung) and three high yielding Tongil(hybrid between Japonica and Indica types) types(Yongmoon, Taeback and Joongwon) -at six different stages of development(4, 8, 12, 16, 20 and 32 days after flowering(DAF)) were kindly supplied by the Agricultural Experimental Station, Suwon, Korea. The grains were dried overnight in an air-oven(60°C), dehulled mannually, ground by a mortar and pestle to pass through 60 mesh screen. The ground sam-

ples were defatted with cold acetone in a Soxhlet apparatus for 20 hr, dried under hood for several hrs and subsequently in an air-oven (102°C) for 3 hr and stored at 5°C until later use.

Crude protein

Total nitrogen was determined by micro Kjeldahl method and a conversion factor of 5.95 was used for conversion to crude protein.

Extraction of proteins for 2-D electrophoresis

A procedure of Ames and Nikaido was modified as follows. The rice sample (0.5g) with 5 ml of 2% SDS/5% 2-mercaptoethanol(2-ME) solution was stirred at room temperature for 30 min and centrifuged at 17,000×g for 20min. The supernatant was heated for 5min in a 98°C water bath and then immediately cooled in an ice bath. Protein contents of the extract were determined with a Bio-Rad Protein Assay Kit and immediately applied to the isoelectrofocusing gel.

Molecular weight standards

Phosphorylase B(92,500), bovine serum albumin(66,200), ovalbumin(45,000), carbonic anhydrase(31,000), soybean trypsin inhibitor(21,500) and lysozyme(14,400) were used as molecular weight standards.

2-D Electrophoresis

The procedure of O'Farrell[11] was modified as follows. Isoelectrofocusing(IEF) was done in groups of 6 simultaneous cylindrical gels(13cm long with 0.3cm diameter) using a LKB 2117 Electrofocusing Unit. The IEF gel mixture was prepared according to O'Farrell[11] except that 15 μl 10% ammonium persulfate and 10μl N,N,N′,N′-tetramethyleneethylenediamine (TEMED) were added. The extractant containing between 500 and 550μg protein was loaded. The electrofocusing was carried out to equilibrium at 150V for 1hr, at 300V for 22hr and then at 450V for 1hr. The pH gradient across each gel was measured as described by Ames and Nikaido.[12] Six slab gels(10% acrylamide) were simultaneously used for the second dimension separations using a Bio-Rad PROTEIN 11 Multi cell at 19°C. The gels were stained with Coomassie Brilliant Blue.

Results and Discussion

Crude protein content

As grain ripened, crude protein contents of all Japonica types and Yongmoon showed continuous decreases until 20 days after flowering (DAF) followed by slight increases at 32 DAF (Fig. 1). In case of Joongwon and Taebaek,

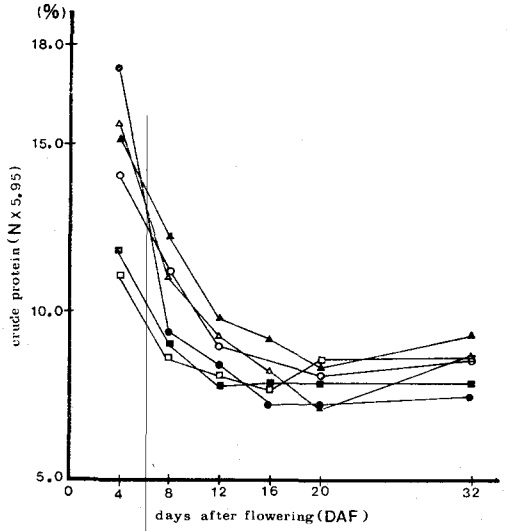

Fig. 1. Crude protein content of dehulled developing rice grains from six varieties(○, Sobaek; ●, Sangpoong; △, Choochung; ▲, Yongmoon; □, Taebaek; ■, Joongwon)

crude protein contents were continuously decreased until 12 and 16 DAF, respectively, followed by subsequent slight increases. Cagampang et al.[6] and Villareal and Juliano[7] also reported that crude protein contents of rice varieties they investigated showed decreases until 32 and 21 DAF, respectively.

Extraction rates of proteins

Since maximum protein extraction was desired for subsequent 2-D electrophoresis, several ext-

ractants such as sodium chloride solutions(0.7 M, 1.0M and 1.7M), 2% SDS/2% 2-ME, 2% SDS/5% 2-ME and 5% SDS were compared for their extraction efficiencies of proteins from dehulled rices(purchased from a local market) (the extraction time was 30min in all cases) and 2% SDS/5% 2-ME gave the highest extraction rate 93.4%. Therefore, 2% SDS/5% 2-ME was chosen as the extractant for the experiment. This result was compared favorably with that of Juliano and Boulter.[13] who reported extraction rate 94% when rice was extracted with 0.5% SDS/0.6% 2-ME for 1~2hr.

The extraction rates of rice samples varied greatly from 37.4%(4DAF, Yongmoon) to 99.3 %(20 DAF, Sangpoong) and they were generally lowest in early DAF with increases as seed developed(Table 1). It was noteworthy that Yongmoon had particularly low extraction rates (less than 74.9% at all DAFs).

Table 1. Rice protein extraction rates* of six varieties at six different stages of development extracted with 2% sodium dodecyl sulfate/5% 2-mercaptoethanol(%)

Varieties	Days after flowering					
	4	8	12	16	20	32
Sobaek	76.2	52.0	84.0	NA[a]	86.5	71.5
Sangpoong	51.7	64.4	85.6	96.7	99.3	95.7
Choochung	51.2	80.1	90.6	91.8	90.9	83.9
Yongmoon	37.4	56.6	56.2	60.0	74.9	71.6
Taebaek	70.0	77.7	89.2	94.9	87.6	86.9
Joongwon	60.5	69.2	98.4	74.3	74.3	82.1

[a]NA : Not available because the sample was not collected at the due time of collection.
* Mean value

2-D Electrophoresis

The pH gradient of first-dimension isoelectric focusing gels was linear in the range of pH 5.2 ~8.5(Fig. 2). Effective molecular weight range was 20,000~100,000. A typical 2-D pattern is shown in Fig. 3, which is that of proteins extracted from Taebaek at 12 DAF. Most of the polypeptide spots were in pH range of 6.0~8.4 and in the molecular weight range of 30,000~ 93,000.

Fig. 4 is the composite 2-D map of rice pro-

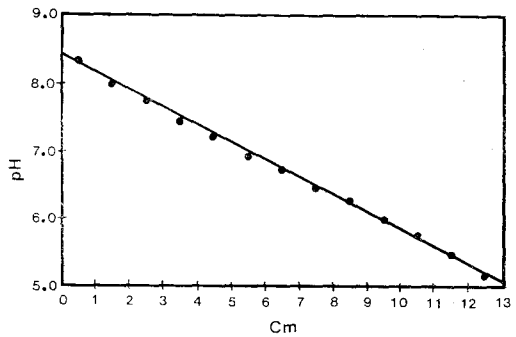

Fig. 2. Standard pH gradient for isoelectrofocusing

Fig. 3. A typical two-dimensional gel electrophoresis map of rice seed proteins(from Taebaek at 12 days after flowering)

teins, constructed by analyzing 2-D electrophoretic patterns of the six varieties at all (six) stages of seed development. More than 300 polypeptide spots were observed and they were divided into 7 groups (a~g)(employed for descriptive purposes only) and the spots in each group were numbered; e.g., a1, a2, *etc.*

Identification of the spots needs to be done in the future using various techniques listed in reference 14 and no attempt has been made to identify them in the present paper. However, polypeptides in f group are most probably the

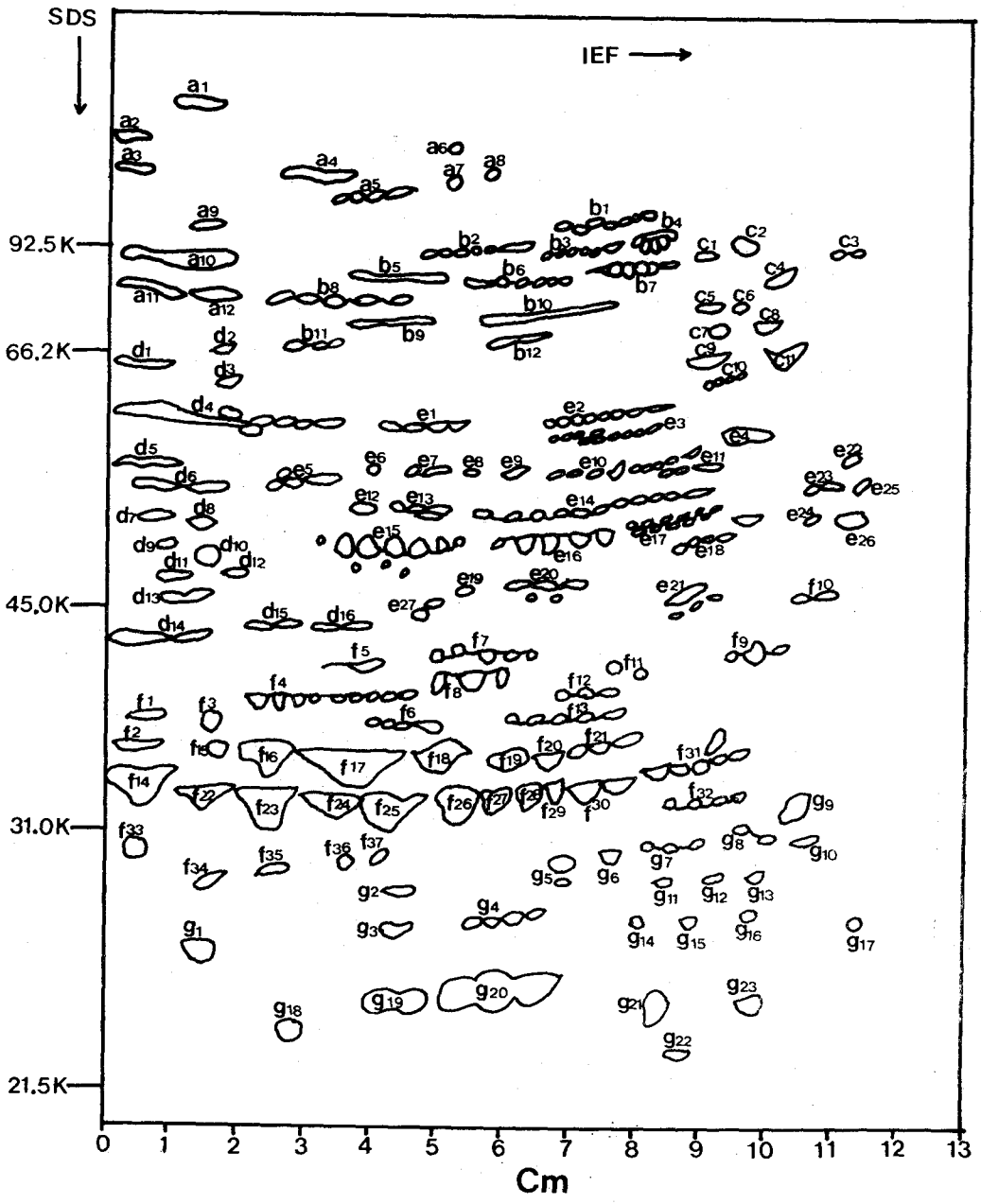

Fig. 4. Composite two-dimensional gel electrophoresis map of rice seed proteins of six varieties from 4 to 32 days after flowering

subunits of rice glutelins, since they are the most prominent spots in all the samples(the glutelins may constitute up to 75% of the grain protein)[7] and the molecular weight of a major rice glutelin subunit is 34,000~38,000[7,10]. There were many spots which might be related; those such as e2, e3, e14 and f4 lying along horizontal lines which may be different by one or more charges and those such as e3 and e17 lying fairly close along a vertical line which may be related to mass variants involving additions or deletions.

At 4 DAF, the spots f14~f31 were the major and the only(in most varieties) spots visible and were not separated as clearly as in the more developed grains.

At 8 DAF, the intensity and the number of spots were increased significantly, more in case of Tongil type varieties compared to Japonica type varieties, indicating earlier development of Tongil type varieties. In Taebaek and Joongwon, the major spots in f group were bigger and better separated than in Yongmoon, Sobaek, Sangpoong and Choochung.

At 12 DAF, the intensity and number of spots increased in Sobaek, Sangpoong and Choochung and spots in the molecular range above 45,000 appeared in all varieties. Especially in Sangpoong, the spots in b, c and e groups appeared and the spots in f and g groups were well separated. Taebaek at 12 DAF showed more number of spots than any other DAF. Proteins of high molecular weight above 100,000 appeared at 12 DAF in all varieties as indicated by the presence of proteins at the top of separating gel.

At 16 DAF, Yongmoon and Joongwon showed more number of spots than at other DAF and characteristically had well separated spots in c, e and g groups in the pH range 5.5~6.5.

Each variety showed characteristic maturity profile of polypeptide spots; i.e., different varieties exhibited maximum number of spots at different DAFs. Taebaek peaked at 12 DAF and then declined. Yongmoon and Joongwon peaked at 16 DAF and then declined. Sobaek peaked at 12 DAF with no further substantial change of number of spots as rice was matured. Choochung and Sangpoong continued to increase the number of spots until full maturity at 32 DAF.

Some of the spots were present exclusively in one or more of the varieties; spots a1, a2, a3, a5 and e18 only in Taebaek, a6, a7, a8, c3 and e22~26 only in Sangpoong, b10 and b12 only in Taebaek and Sangpoong, c1 only in Joongwon and Taebaek, c6 only in Yongmoon, c12 only in Joongwon and Choochung, d12 only in Joongwon, f6 in all but Choochung and Sobaek, g6, g8 and g12 only in Taebaek, Yongmoon and Joongwon. Spots f16, f17, f18, f23 and f24 were present in all the varieties at all DAFs.

In conclusion, 2-D electrophoretic analysis of rice seed proteins revealed vast differences in the number, kind and intensity of the polypeptides dependent on variety and maturity, and the polypeptides needed to be identified. The information obtained would play a vital role in elucidating the relationship between genes and functions.

Abstract

High resolution two-dimensional (2-D) electrophoresis with isoelectrofocusing in the first dimension and electrophoresis in sodium dodecyl sulfate in acrylamide gradient gels in the second dimension has been used to produce maps of proteins, extracted from rice seeds with 2% sodium dodecyl sulfate/5% 2-mercaptoethanol. Six rice cultivars-three Japonica types and three Tongil(high-yielding) types-at six maturities were studied. Composite map was constructed and more than 300 polypeptide spots were counted in the pH range of 5.2~8.3 and molecular range of 20,000~100,000. Vast differences were observed between varieties and between maturities in the maps.

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