

Note

Screening of Rice Strains Deficient in 16-kDa Allergenic Protein

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Most rice contains the 16-kDa allergenic protein belonging to the α -amylase/trypsin inhibitor family. The amount of this protein in more than 150 rice strains was examined by using the antibody raised against the 16-kDa allergenic protein. While all of Japanese cultivars tested contained nearly same amount of the 16-kDa allergen, some strains in other Asian countries contained little or none of this protein.

It has been reported that cereal grains contain allergenic proteins.^{1–6)} Rice also contains allergenic proteins which are classified into albumin and globulin based on their solubility properties. Rice endosperm contains 0.3–9.9% albumin of the total seed protein.⁷⁾ The major albumin of japonica rice, showing heterogeneity in molecular weight (14,000–16,000) and isoelectric point (pI 6–8), has been characterized as an antigenic protein to the IgE antibody of allergic patients.⁴⁾ One of these rice allergenic proteins (RA) has been isolated, cDNA encoding RA being recently cloned and its nucleotide sequence determined.⁸⁾ The primary structure of RA deduced from the nucleotide sequence showed homology to a wheat α -amylase inhibitor, suggesting that RA might be a member of the α -amylase/trypsin inhibitor family in cereals.^{8,9)}

Although rice is the main crop in Asia, allergic patients have to omit rice from their meals, because to date, there is no good therapy yet for rice-allergic patients. A rice strain deficient in allergenic proteins might be very useful for rice-allergic patients, if one exists. Therefore, we investigated rice varieties to identify any which do not contain RA. One hundred and twenty nine varieties of the *O. sativa* family and 19 of wild-type species were provided by International Rice Germplasm Center of International Rice Research Institute (IRRI), and ten species of Philippine cultivars were provided by Philippine Rice Research Institute. Japanese cultivars were presented by Dr. T. Tashiro (Nagoya University). Mouse monoclonal antibody 25B9 raised against 16-kDa allergen had been obtained previously.¹⁰⁾ More than 100 strains were screened by SDS-PAGE and immunoblotting, SDS-PAGE being performed as described by Laemmli.¹¹⁾ To visualize the proteins, Coomassie Brilliant Blue R-250 was used. An immunoblotting analysis of the rice proteins was performed as described by Towbin *et al.*,¹²⁾ after SDS-PAGE. To visualize the proteins crossreactive with anti-RA-mAb, anti-mouse IgG conjugated with horseradish peroxidase and the chromophore, 4-chloro-1-naphthol, were used. Rice proteins were extracted from three dehulled milled grains by 200 μ l of phosphate-buffered saline with ultra-sonication. After removing the debris by centrifuging, 10 μ l of each supernatant was applied for SDS-PAGE. This procedure enabled albumins and globulins to be predominantly extracted. Most of the cultivars contained a relatively large amount

of RA by the immunoblotting analysis (data not shown). Figure 1 shows some results of the SDS-PAGE (A) and immunoblotting analyses (B) for several rice cultivars. Compared with japonica cv. Nipponbare (lane 1), three cultivars (lanes 3, 4, and 5) of GEMJYA JYNAM (Butan), MATIA AMAN 53-13 (Bangladesh), and TAPOL (Philippines) contained relatively less 14–16-kDa albumin. All of them are cultivated in Southeast Asia. In addition, screening was done on many Japanese cultivars exhibiting an almost identical protein pattern by SDS-PAGE, but there was no cultivar having no RA (data not shown). These results could be due to the fact that most Japanese cultivars are sister-line varieties derived from genetically close species. Some results for the wild-type species are shown in Fig. 2. While *O. nivara*, *O. latifolia*, and *O. rufipogon* (Bangladesh) contained RA (lanes 1, 4, 6, 13, and 16 in Fig. 2), other strains contained no detectable amount of RA by immunoblotting. Thus, some cultivars and wild-type species were found to contain little or almost no RA in their grains. Rice grains of these cultivars might be useful for rice-allergic patients in Japan as substitutes for japonica rice, although no or low allergenicity should be confirmed by clinical tests such as RAST. Most of the

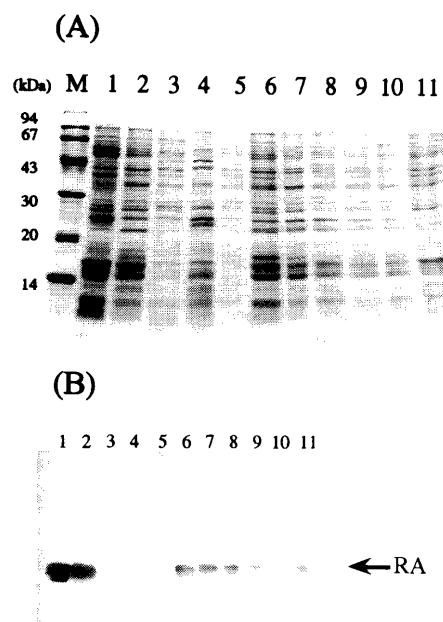


Fig. 1. SDS-PAGE (A) and Immunoblotting (B) Analyses of Rice Cultivars.

Immunoblotting was performed by using mAb 25B9 (B). 1, NIPPONBARE; 2, BARAN BORO; 3, GEMJYA JYNAM; 4, MATIA AMAN 53-13; 5, TAPOL; 6, BAN-HO; 7, PTB21; 8, BHADOIA 233; 9, KURUHONDAWALA; 10, GANGALA; 11, SADONG.

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Abbreviation: mAb, monoclonal antibody.

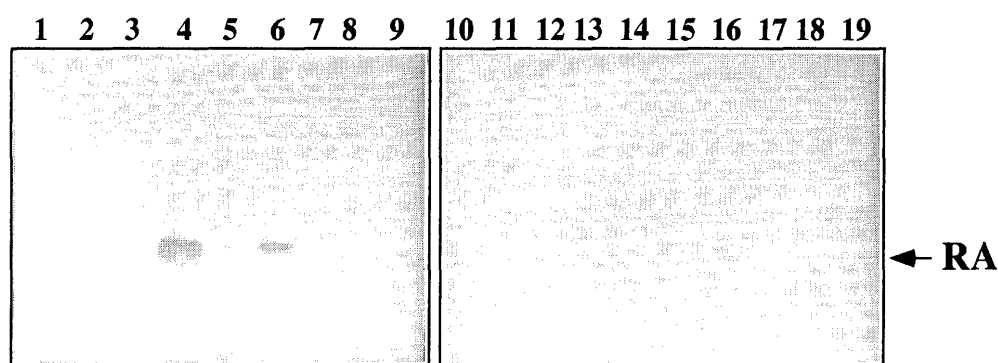


Fig. 2. Immunoblotting Analysis of Wild-type Rice Species.

1, *O. latifolia* (Costa Rica); 2, *O. rufipogon* (India); 3, *O. officinalis* (Thailand); 4, *O. rufipogon/O. nivara* (China-Taiwan); 5, *O. rufipogon* (China); 6, *O. nivara* (Cambodia); 7, *O. punctata* (Ghana); 8, *O. minuta* (Philippines); 9, *O. minuta* (U.S.A.); 10, *O. punctata* (Ghana); 11, *O. rufipogon* (India); 12, *O. officinalis* (Indonesia); 13, *O. nivara* (Sri Lanka); 14, *O. latifolia* (Argentina); 15, *O. australiensis* (Australia); 16, *O. nivara* (Sri Lanka); 19, *O. rufipogon* (Nepal). The same name may exist, but seed sources were different.

wild-type species have poor endosperm, and this might hamper their eating quality compared with the cultivars. Improvement of the seed proteins of these wild-type rice species will be needed. Research work to remove the allergenic proteins from rice grains of a Japanese cultivar by the gene-manipulation technique is also in progress.

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