Note

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Analysis of Rice Proteins of Transgenic Rice Resistant to Rice Stripe Virus

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Genetically engineered plants that are resistant to diseases and harmful insects have been developed [1-3]. Such plants are believed to increase the yield of such crops. Rice stripe virus (RSV), one of the tenuiviruses transmitted by the small brown planthopper, causes severe damage to rice in Japan [4]. Hayakawa *et al.* have developed a genetically engineered rice resistant to RSV [5]. They induced the coat protein gene of RSV into a japonica variety of rice to establish RSV-resistant rice. The first test for safety assessment of the plant in the environment was done in an isolated growth environment.

One of the next steps in the safety analysis of the transgenic rice would be an analysis of the changes in the properties of allergens of the transgenic crops [6]. In this study, we analyzed the patterns of the proteins of RSV-resistant rice and a host strain Kinuhikari to show the properties of a 16 kDa rice allergen of the transgenic rice.

The RSV resistant strain of Kinuhikari (TR8-3) established by Hayakawa *et al.* [5] and non-transgenic host strain Kinuhikari were harvested in 1996 in the test field of the Plant Research Institute (Yokohama, Japan). The rice seeds were threshed and stored at 4°C until use. Rice seeds were ground and the resulting rice powder was used. Rice powder (10 mg) was suspended in deionized water (90 μl) and mixed with an SDS-sample buffer (100 μl) containing 2-mercaptoethanol. The mixture was heated at 90°C for 5 min and centrifuged at 15,000 rpm for 15 min. The supernatant was used as the whole rice proteins. Salt soluble protein fractions including the 16 kDa rice allergenic protein were extracted according to the method of Matsuda *et al.* [7]. Rice powder (2 g) was mixed with 0.5 M NaCl (20 ml) and extracted by stirring for 2 hr at room temperature. The mixtures were centrifuged at 10,000 rpm for 30 min, and the supernatants (18 ml) were obtained. Saturated ammonium sulfate solution (18 ml) was added to the supernatants and centrifuged. Precipitates were dissolved with deionized water (5 ml) and dialyzed against 20 mM Tris-HCl buffer (PH 8.6). Precipitates were then removed by centrifugation.

The whole rice proteins were analyzed by an SDS-PAGE [8] under reduced conditions using a 10 to 20% gradient gel (Daiichi-kagaku, Japan). The proteins on the gel were stained by the Quick-CBB dye method (Wako Pure Chemicals, Japan). **Fig. 1** shows the

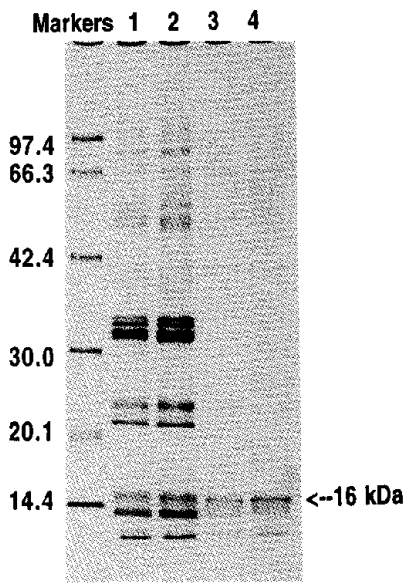

Fig. 1 SDS-PAGE analysis of rice proteins of transgenic rice. The whole rice proteins (lane 1; Kinuhikari, lane 2; TR8-3) and salt soluble protein fractions (lane 3; Kinuhikari, lane 4 ; TR8-3) were analyzed by an SDS-PAGE under reduced conditions using a 10 to 20% gradient gel. The proteins on the gel were stained by the Quick-CBB dye method. Lysozyme (14.4 kDa), trypsin inhibitor (20.1 kDa), carbonic anhydrase (30.0 kDa), aldolase (42.4 kDa), bovine serum albumin (66.3 kDa), and phosphorylase b (97.4 kDa) were used as standard marker proteins.

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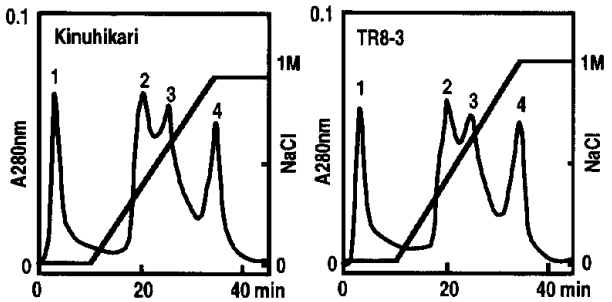

Fig. 2 Ion-exchange chromatography of salt soluble proteins of transgenic rice.

The salt soluble protein fractions were charged on a Hi-Q column equilibrated with 20 mM Tris-HCl buffer (PH 8.6). The column was eluted with NaCl gradient.

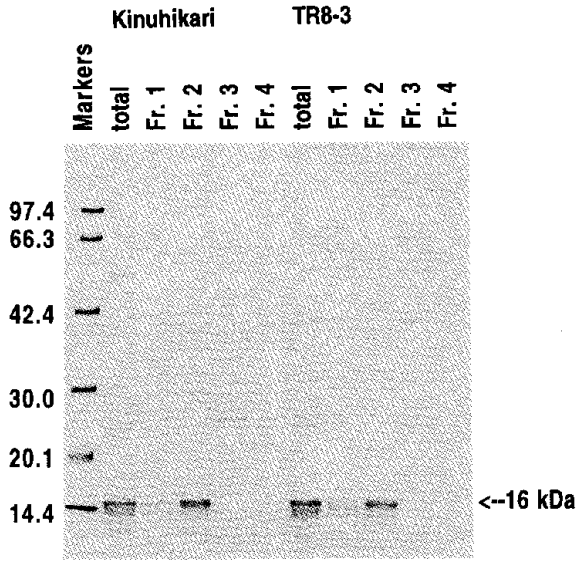

Fig. 3 SDS-PAGE analysis of the salt soluble proteins separated by ion-exchange chromatography.

The salt soluble proteins separated by ion-exchange chromatography were analyzed by an SDS-PAGE under reduced conditions using a 10 to 20% gradient gel.

result of SDS-PAGE analysis of whole rice proteins. Lane 1 shows the result of the extracts from Kinuhikari and lane 2, those from TR8-3. The two specimens yielded the same profiles of the PAGE patterns. Lane 3 and 4 were the results of the salt soluble protein fractions from Kinuhikari and TR8-3. The major protein bands detected were the 16 kDa rice allergen as reported by Nakamura and Matsuda [9]. Additional lower molecular weight bands and light bands with higher molecular weight (23 kDa, 34 kDa, 40 kDa, 50 kDa, 57 kDa and 65 kDa) were also observed.

The salt soluble protein fractions (0.5 m*l*) were charged on a Hi-Q column (bed volume of 1 m*l*, Bio-Rad, USA) equilibrated with 20 mM Tris-HCl buffer (PH 8.6). The column was eluted with NaCl gradient (0 to 1 M NaCl). **Fig. 2** shows the elution profiles of the salt soluble protein fractions from Kinuhikari and TR8-3. The two elution profiles seem to be similar; no additional peak was observed.

Fig. 3 shows the result of SDS-PAGE of the Hi-Q fractions. The 16 kDa allergenic protein was eluted in fractions 1 and 2. Whereas some higher molecular weight bands seemed to differ in concentration, there seemed to be no difference in the 16 kDa bands between Kinuhikari and TR8-3.

From the above results, we concluded that Kinuhikari and the genetically engineered RSV-resistant strain of Kinuhikari (TR8-3) contained the 16 kDa rice allergenic protein with the same properties. The results suggested that induction of the coat protein gene of RSV did not affect the expression of the 16 kDa allergenic protein. The 16 kDa allergenic protein is one of the major allergens of rice. The protein is thought to be a member of α-amylase/trypsin inhibitors and believe to have very rigid structure because of its high cystine content [9].

Rice is also known to contain another type of allergenic protein as detected in sera from patients with rice allergies [7]. Sera from those patients seemed to be very useful for analysis of the food allergens. Further analysis with that sera would be very important to clarify the allergenicity of the transgenic rice.

References

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