

Note

Primary Screening of Relatively Less Allergenic Wheat Varieties

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Summary Since the number of wheat-allergic patients has been increasing in recent years, less allergenic wheat varieties are socially demanded. The aim of this study was to primarily screen 324 varieties of wheat picked up from various parts of the world. Prior to the screening, the inhibition ELISA (enzyme-linked immunosorbent assay) was examined with major wheat allergens (glutenin, gliadin and α -amylase inhibitor). As a result, the patient's IgE antibodies used in this assay reacted to these three allergens, and thereby turned out to be suitable for the primary screening for the less allergenic wheat varieties. Thus, the allergenicity of each wheat sample was measured by direct ELISA using the sole patient's serum as an IgE source and 'Aoba Komugi' as a control (a value of 1.0). The mean $\pm$ SD of all kinds was 1.14 ± 0.37 . Among them, 'CM32859' (Mexico) gave the lowest value (0.38). Several other varieties, such as 'Einkorn' (Unknown), 'Iliniaza' (Ecuador), 'Hsin Mai 2' (China), 'Italy 1' (Italy) and so on, also serve as candidates for the less allergenic varieties. More detailed secondary screening will lead to a finding of the less allergenic wheat varieties for wheat-allergic patients, because these varieties are expected to become mother plants for breeding.

Key Words wheat allergen, ELISA, less allergenic variety

Food allergies have become one of the most important health problems in recent years, and wheat flour is a common allergy-inducing food throughout the world. We have already developed a method of producing hypoallergenic wheat flour on an industrial scale by treatment of food-usable enzymes (1–4). In the meantime, wheat varieties of less allergenicity are demanded for wheat-allergic patients. In this study, by using a wheat-allergic patient's IgE-ELISA (enzyme-linked immunosorbent assay), we screened for relatively less allergenic wheat varieties from 321 kinds of varieties which were kindly provided from the National Institute of Agrobiological Sciences (Tsukuba, Japan), Hokkaido Prefectural Plant Genetic Resources (Hokkaido, Japan) and other prefectural experiment stations. These 321 varieties included *Triticum aestivum* (L.) Thell., *Triticum compactum* Host., *Triticum durum* Desf., *Triticum dicoccum* (Schränk.) Schubl., *Triticum monococcum* L., *Triticum polonicum* L., *Triticum spelta* L., *Triticum turgidum* L. (see Table 1).

At first, using ten patients' individual sera, we evaluated whether or not the sera recognized nearly all kinds

of major wheat allergens, that is, glutenin (Tokyo Kasei Kogyo Co., Japan), gliadin (MP Biomedicals, US) and α -amylase inhibitor (from wheat, Wako Pure Chemical Industries, Japan), by the inhibition-ELISA method (4–6). The inhibition ELISA was performed as follows. A wheat grain (Aoba Komugi) was crushed, and the broken grain (about 40 mg) was transferred into the bottom of the centrifugation tube (15 mL). Then, 4 M Urea containing 0.1 M Tris-HCl buffer (pH 8.6) (UTH buffer, 5 mL) was added to the tube, and the allergens were extracted by overnight vigorous shaking. The extract was obtained by centrifugation at $1,300 \times g$ for 5 min. Aliquots (100 μ L) of the obtained extracts were coated onto wells and left overnight at 25°C. Unoccupied sites in the wells were blocked with 200 μ L of Block-Ace (Dainippon Pharmaceutical Co., Japan). Separately, glutenin, gliadin, and α -amylase inhibitor were dissolved in PBS (1–1,250 μ g/mL at the final concentration). Each antigen solution (400 μ L) was added to diluted serum samples (100 μ L). After 2 h of preincubation at 25°C, the antigen-serum mixture (100 μ L) was added to the above-mentioned microtiter plates (96 wells, MaxiSorp, Nunc, Denmark). Serum that was not treated with allergens was used as a control. The binding of IgE-antibody to wheat flour was determined by sequential incubation with biotinylated anti-human IgE (Vector Laboratories, US), streptavidin-peroxidase conjugate (Roche Diagnostics, Switzerland), and *o*-phenylenediamine (Wako Pure Chemical Industries). The wells were washed at each step by the addition of PBS containing 0.02% Tween-20 (PBS-T). The values in Fig. 1 are expressed as relative ELISA values based on the

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absorbance at 450 nm.

By this screening, the serum (patient number, TS8699) from International Bioscience (Tokyo, Japan) was chosen as the most suitable one, since, as shown in Fig. 1, the binding of IgE-antibody to plate-coated wheat extracts was inhibited to about the 50% level by preincubation with all three allergens, that is, glutenin, gliadin or α -amylase inhibitor. Among the three allergens, α -amylase inhibitor was the most potent for the patient since the ELISA inhibition was the most prominent even at lower concentrations. α -Amylase inhibitor is known as a contributing allergen to both asthma and urticaria (7, 8) although its content in wheat flour is rather low.

Gluten, the major wheat protein fraction, is composed of glutenin and gliadin fractions. In this study (Fig. 1), glutenin gave the highest ELISA-inhibition at the concentration of 1,250 $\mu\text{g/mL}$. We have already reported that the major wheat allergen is low-molecular weight glutenin and that the repeated sequence, Gln-Gln-Gln-Pro-Pro, in low-molecular weight glutenin is one of the epitope structures for IgE-binding (4). Furthermore gliadin inhibited the binding of IgE-antibody to the plate-coated wheat extract, while the inhibition was the weakest among three allergens.

From the results of inhibition-ELISA, it was judged

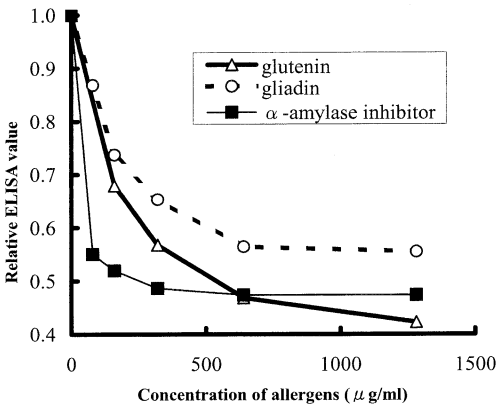


Fig. 1. Reactivities of the patient's IgE against gluten, gliadin and α -amylase inhibitor. The inhibition ELISA was performed as follows. Briefly, the extracts of a wheat grain were coated onto the wells of microtiter plates. Separately, glutenin, gliadin, and α -amylase inhibitor were pre-incubated with serum samples. The antigen-serum mixture was added to the above-mentioned wells. Serum not treated with allergens was used as a control. The binding of IgE-antibody to plate-coated wheat was determined by sequential incubation with biotinylated anti-human IgE, streptavidin-peroxidase conjugate, and *o*-phenylenediamine. The values are expressed as relative ELISA values based on the absorbance at 450 nm. The patient's IgE antibodies used in this assay reacted to these three allergens.

Table 1. Allergenities of wheat grains expressed as the relative ELISA value (REV), and their protein contents.^a

From lowest varieties			From highest varieties		
	REV	Protein (%)		REV	Protein (%)
1 Aoba Komugi (Shizuoka) ^b	1.00	10.5	1 Turkey	2.72	12.8
1 (Hua(R)(2)×	0.38	9.4	2 Toukai 55	2.61	12.3
MY54-N10B/NAPO) MUS“S”			3 Toyokuni	2.46	14.0
CM32859-1M-1Y-1M-0Y			4 Vagued Epis	2.29	10.0
2 Italy 1	0.48	15.9	5 Touzan 16	2.28	10.3
3 Iliniaza	0.52	13.3	6 Tob 8156(R)	2.24	11.3
4 Einkorn ^c	0.54	8.5	7 Soren (USSR) Komugi 490	2.21	9.6
5 Hsin Mai 2	0.57	9.8	8 Sevenno	2.18	11.0
6 Kounosu 25	0.58	12.6	9 Toranoo	2.16	13.5
7 Banner Berkley	0.58	9.4	10 Suyuk 18	2.14	12.2
8 Nanryuu Komugi 1 ^d	0.59	10.0	11 YD“S”[(FN-TH-(3)×	2.11	10.2
9 Syunyou	0.60	8.0	1144.29-TH(2)/CFN(4))SR]		
10 Haruhikari	0.61	12.2	CM35735-11M-2Y-1		
11 Nourin 61 (Shizuoka)	0.62	8.9	12 Rubin	2.06	11.5
12 (TH(6)-KF×	0.63	10.4	13 Waseaka	2.02	11.7
LEE(6)-KF/CAL)ALD“S”			14 Iraq Komugi	1.94	14.3
SWM3410-1M-1Y-1M-1Y-4M-2Y-			15 C.I.12633	1.88	10.7
13 80.1559 Songlen/Selkirk	0.64	12.4	16 Nourin 74	1.88	7.5
14 Loosdorf Winter	0.66	13.9	17 Nisu	1.87	11.4
15 Najah	0.67	12.5	18 Soren (USSR) Komugi 33586	1.87	9.1
16 Aleph	0.68	8.7	19 STW.59/5530	1.87	8.2
17 K338 Etoile de Choisy/Koudie	0.68	10.0	20 Tenmarq	1.85	10.3
18 (BB“S”-Inia(2)×	0.68	7.8			
II20350)(2)F2					
CF283-106R-1M-5R-OM					
19 Banacanka 1	0.69	11.7			
20 G 54327-112	0.70	8.7			

^a Protein content was measured using bovine serum albumin solution as a standard, and indicated as% content in the grain, ^b Source, ^c Einkorn wheat, ^d Rivet wheat.

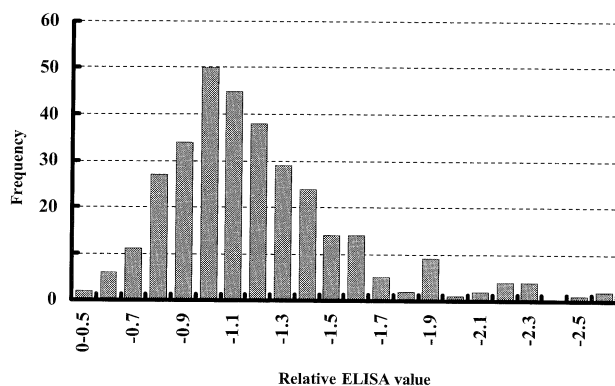


Fig. 2. Histogram of relative ELISA values. The histogram of the allergenicity of 321 varieties (324 kinds) is shown, 'Aoba-Komugi' being used as a control (a value of 1.0). The mean $\pm$ SD for all kinds was 1.14 ± 0.37 .

that the patient's IgE antibody used reacted to nearly all kinds of wheat major allergens. Therefore, this IgE-antibody was thought to be suitable for primal screening of less allergenic wheat varieties from widespread sources. Obviously, it might be better to perform the assay using greater numbers of patients' sera. Practically in Japan, however, obtaining serum (over 100 mL) from one patient was very difficult for the ethical and practical reasons. Pooled serum from several patients seems to be unsuitable since it would be difficult to re-constitute as the same quality when it would be once finished. For these reasons, we obtained about 100 mL of serum from International Bioscience with the donor's informed consent.

Next, we compared the allergenicity of 321 varieties (324 kinds) using the direct-ELISA method. The extracts were prepared from each variety using UTH buffer as mentioned above. The resulting extracts (100 μ L) were coated onto the wells and left overnight at 25°C; unoccupied sites in the wells were blocked with 200 μ L of Block-Ace for 1 h at 37°C. The 1 : 15 diluted serum (100 μ L) was added to the wells and incubated for 1 h at 25°C. This was followed by the addition of biotinylated anti-human IgE, streptavidin-peroxidase conjugate, and *o*-phenylenediamine. The wells were washed with PBS-T. Allergenicity was expressed in arbitrary units based on the absorbance at 450 nm. The assay was performed at least two times using 3–6 grains for each, and the values shown in Table 1 are the means. CV (coefficient of variation) in this assay was usually less than 12.5%.

Furthermore, the protein content of each extract from wheat grains was measured with a Protein Assay Rapid Kit (Wako Pure Chemical Industries). This assay is based on the pyromolex coloring method, and protein content was calculated as % content in the grain using bovine serum albumin solution as a standard. In this assay, urea solution less than 6 M did not affect the measurement.

Since it was difficult to assay everything in one assay, we divided these 324 kinds into 11 groups. Instead, in every assay for each group, 'Aoba Komugi' was used as a control between different assays. Surprisingly, as a

result, there were found notable differences in the relative ELISA value between wheat varieties, ranging from 0.38 to 2.72 (Table 1). The mean $\pm$ SD for all kinds was 1.14 ± 0.37 (Fig. 2), in which 'CM32859' (Mexico) gave the lowest value (0.38). 'Einkorn' (Unknown), 'Iliniaza' (Ecuador), 'Hsin Mai 2' (China), and 'Italy 1' (Italy) gave relatively lower values than others in common with several repeated assays. Moreover, the varieties with less allergenicity didn't have a tendency to have less protein. Further studies are necessary to clarify the relationship between allergenicity and the content of each allergen.

Among Japanese varieties, the relative ELISA values for 'Kounosu 25,' 'Syunyou,' 'Haruhikari,' and 'Nourin 61' were nearly the same as those for the above-mentioned foreign varieties. As for 'Nourin 61,' its allergenicity varied somewhat with the place of cultivation (data not shown).

In conclusion, we provided here a method of screening less allergenic wheat grains. Among 324 wheat varieties tested, several varieties, such as 'CM32859,' 'Einkorn,' 'Iliniaza,' 'Hsin Mai 2,' 'Italy 1' and so on, were selected as candidates for less allergenic varieties. Detailed analyses of them (the second screening) are now being carried out using an enlarged number of patients' sera.

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