

REPORT

Immunological study of different fraction of wheat proteins

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Abstract: Wheat allergy specifically refers to the adverse reaction involving IgE antibody to one or more protein fraction of wheat such as albumin, globulin, gliadin and glutenin (gluten). The majority of IgE-mediated reactions to wheat involve albumin and globulin fraction while gluten (gliadin & glutenin) also cause allergy (Celiac disease). Allergic reactions to wheat may be caused by ingestion of wheat containing foods or inhalation of flour (Bakers asthma). The present study was an effort to explore the antibody response of different proteins present in wheat. ELISA results revealed that the antibody response for albumin varied from 0.92-1.78, whereas, for globulin ranged from 1.39-1.60. Antibody response against glutenin and gliadin ranged from 0.57-1.05 and 0.98-1.95 respectively, among the different varieties of wheat. All the tested wheat varieties showed the significant difference antibody response against the different fractions of protein.

Keywords: Albumin, antibody, immunized, ELISA, Celiac disease.

INTRODUCTION

The principal allergenic substance in wheat that engage the allergic reaction to the body is mostly protein, humans eat large numbers of proteins but among these proteins only minute proportions are allergens, because these proteins possess specific intrinsic features of allergenicity (Huby *et al.*, 2000). The allergic response to cereal grain of the sera from atopic food allergic patients show Immunoglobulin E (IgE) respond with wheat proteins specially salt soluble proteins albumin and globulin have a high degree of allergenic activity. Wheat grain proteins have been concerned in both respiratory and food allergies, as well as hypersensitivity. Different fractions of wheat grain contain various allergens, these allergens may be lipid transfer protein and alpha amylase/trypsin inhibitor and these substances are initiated with water and salt soluble fraction of wheat. While LMW-glutenins and omega 5-gliadins are initiated with insoluble portion of protein (gluten) (Battais *et al.*, 2004), there is another form of wheat allergy called coeliac disease caused by ingestion of cereals containing gluten. Gluten made up of two component gliadin and glutenin but gliadin is responsible for coeliac disease, which is isolated from 70% alcohol soluble fraction (Howdle *et al.*, 1984). Several wheat allergens are concerned in baker's asthma 70-80% of allergic reactions have IgE binding activity

(Weiss *et al.*, 1993; Posch *et al.*, 1995). Wheat is the most widely cultivated cereal and represents an important food and dietary protein source worldwide. Wheat proteins can be either ingested or inhaled in the form of raw flour, which can lead to adverse reactions (Battais F *et al.*, 2008).

Wheat is most important sources of food and dietary protein which is widely cultivated throughout the world. The protein present in the wheat can either be taken through the diet (ingested) or inhaled in the form of flour dust (raw flour) may lead the adverse/allergic reactions (Battais F *et al.*, 2008). Tatham *et al.* (2008) described that wheat have been concerned in both allergic response and wheat intolerances (coeliac disease). Moneret-Vautrin *et al.* (2005) reported that 06% of wheat allergens have been involved in life-threatening food anaphylaxis reactions among the year of 2002 and 2004.

MATERIALS AND METHODS

Fourteen wheat (grain) varieties namely Kiran-95, Amber, Sindh-90, Sarsabz, Khirman, Jauhar-18, Mehran-89, Anmol-91, TJ-83, GP-256, GP-205, Marvi-2000, Soghat-90, Local (Unknown), were obtained from official organization, Nuclear Institute of Agriculture (NIA) Tandojam, while one unknown variety was collected from local market.

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Purification of water soluble proteins (Albumin)

Took 1g of wheat powder and incubated the flour with 1ml of 0.5M NaCl overnight, put in ultrasonic extraction (100w for 30 min at room temperature, centrifuged for 20 min at 14000 rpm. Separated the supernatant, frozen and stored at -40°C.

Purification of water soluble proteins (Globulin)

Took residue from albumin extraction, Precipitated (residue) washed three times with water, incubated the residue with 1 ml of PBS over night at room temperature, Put in ultrasonic extraction (For 30 min at room temperature), Centrifuge for 20 min at 14000rpm, Separated the supernatant, Frozen and stored at -40°C.

Purification of insoluble proteins (Glutenin)

Took residue from globulin extract , precipitated and washed with distilled water (3X) then incubated over night with 1 mL with 50% isopropanol, then put in sonicator (100 w for 30 min) after that centrifuged for 20 min at 14000 rpm, separated the glutenin extract frozen and stored at -40°C.

Purification of insoluble proteins (Gliadin)

Took residue from globulin extraction, Precipitate (residue) washed three times with water, Incubated the residue with 1ml 70% ethyl alcohol over night at room temperature, Put in sonicator (For 30min at room temperature), Centrifuge for 20min at 14000rpm, Separated the gliadin extract 3X, Frozen and stored at -40°C.

Production of antibodies

The determination of immunological response against different wheat protein white rabbits were purchased, the rabbit were immunized by the method of Prabhasankar *et al.*, (2002). The rabbit were injected with different fraction of proteins (Albumin, globulin, glutenin and gliadin). First immunization in concentrated Freund's complete adjuvant (250ug of each fraction of wheat proteins (antigen) in 500ml of 16mM acetic acid per rabbit) followed by two further does with 150ug of antigen per rabbit in Freund's incomplete adjuvant 2 and 4 weeks later. Half does were given intradermally and half subcutaneously. Then rabbits were given 2 months rest. 100 uL boosters were given intradermally. After one week blood was collected, and sera were separated and stored at -20°C for further investigation.

RESULTS

For determination of antibody response against the different fraction of wheat we developed ELISA for assessing the antibody response of Pakistani wheat varieties with respect to allergic reaction.

Antibody response against albumin protein presented in table 1 shows the significant variation in antibody

response among the different wheat varieties grown in Sindh. The antibody response against the albumin varied from 0.92 to 1.78. The highest antibody response found in variety Local (1.78) followed by GP-205 (1.74), TJ 83 (1.73), Kiran-95 (1.72), Jauhar (1.72), Mehran (1.70), Sindh-90 (1.69), Anmol(1.68), Khirman (1.67), Soghat (1.65), Sarsabz (1.50), GP-256 (0.99), Amber (0.94), while the wheat variety Marvi (0.92) exhibited the significantly lower response against albumin variety kiran-95 and Jauhar show the statistically significant response against albumin (fig. 1).

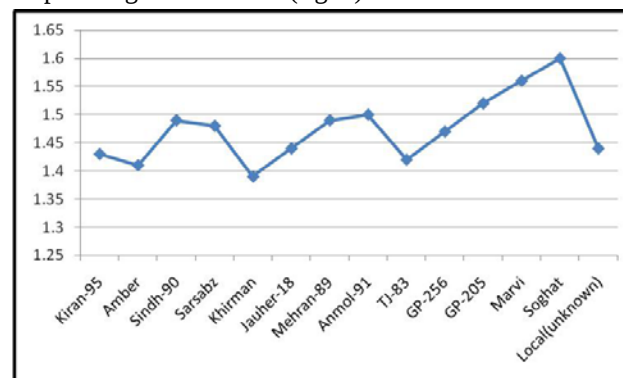


Fig. 1: Antibody response for Albumin

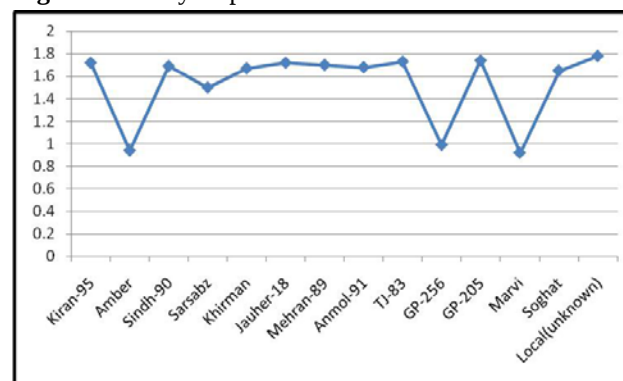


Fig. 2: Antibody response for Globulin

Antibody response against globulin protein varied from 1.39 to 1.60 shown in the table 1, the highest antibody response against the globulin shown in variety Soghat (1.60) followed by Marvi (1.56), GP-205 (1.52), Anmol (1.50), Mehran (1.49), Sindh-90 (1.49), Sarsabz (1.48), GP-256 (1.47), Local variety (1.44), Jauhar (1.44), Kiran-95 (1.43), TJ 83 (1.42) and Amber (1.41). While the variety Khirman (1.39) show the significant low response against the globulin. Significant variation against the globulin seen among the all tested varieties of Sindh except Mehran, sindh-90 and Jouhar, Local shows the statistical similar response against globulin (fig. 2).

It is obvious from the result depicted in table 2 that the antibody response against glutenin ranged from 0.56 to 1.08 among the different wheat varieties cultivated in Sindh. The significant highest response seen in variety Soghat (1.08) followed by TJ 83 (1.02), Khirman (0.96),

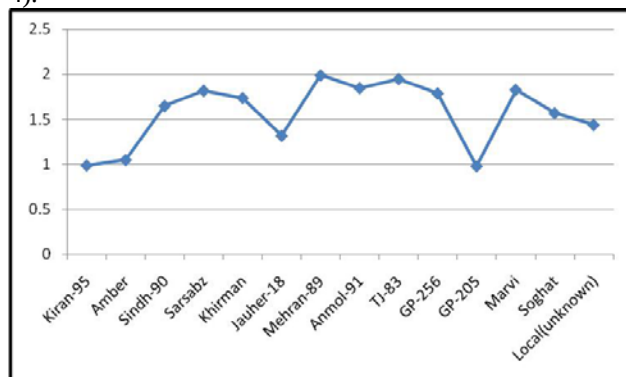
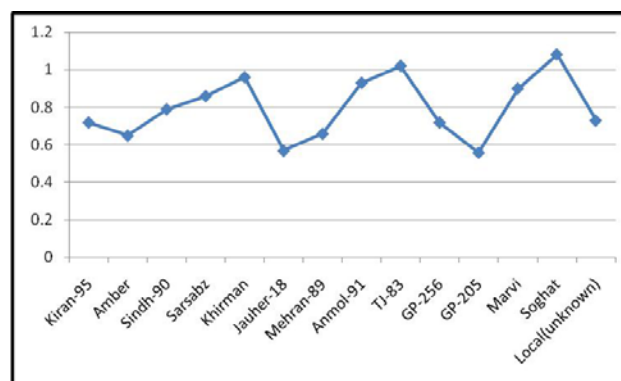
Table 1: Antibody response for Albumin and Globulin

Serial No.	Wheat varieties	Antibody response for Albumin	Antibody response for Globulin
1	Kiran-95	1.72	1.43
2	Amber	0.94	1.41
3	Sindh-90	1.69	1.49
4	Sarsabz	1.50	1.48
5	Khirman	1.67	1.39
6	Jauher-18	1.72	1.44
7	Mehran-89	1.70	1.49
8	Anmol-91	1.68	1.50
9	TJ-83	1.73	1.42
10	GP-256	0.99	1.47
11	GP-205	1.74	1.52
12	Marvi	0.92	1.56
13	Soghat	1.65	1.60
14	Local (unknown)	1.78	1.44

Table 2: Antibody response for Gliadin and Glutenin

Serial No.	Wheat varieties	Antibody response for Gliadin	Antibody response for Glutenin
1	Kiran-95	0.99	0.72
2	Amber	1.05	0.65
3	Sindh-90	1.65	0.79
4	Sarsabz	1.82	0.86
5	Khirman	1.74	0.96
6	Jauher-18	1.32	0.57
7	Mehran-89	1.99	0.66
8	Anmol-91	1.85	0.93
9	TJ-83	1.95	1.02
10	GP-256	1.79	0.72
11	GP-205	0.98	0.56
12	Marvi	1.83	0.9
13	Soghat	1.57	1.08
14	Local(unknown)	1.44	0.73

Anmol (0.93), Marvi (0.9), Sarsabz (0.86), Sindh-90 (0.79), Local variety (0.73), Kiran-95 (0.72), GP-256 (0.72), Mehran (0.66), Amber (0.65), While significantly lower response seen in varieties Jauhar (0.57), GP-205 (0.56). All the wheat varieties show the significant difference except Jauhar and GP205 (fig. 4).

**Fig. 3:** Antibody response for Gliadin**Fig. 4:** Antibody response for Glutenin

It is clear from the result depicted in table 2 that the antibody response against the gliadin ranged 0.98 to 1.99 among the different wheat varieties cultivated in Sindh. The response of antibody against Gliadin presented in table 2, which show the antibody response against the gliadin range varied from 0.98 to 1.95 among the different

wheat varieties cultivated in Sindh. The highest antibody response against gliadin recorded in Mehran (1.99) followed by TJ 83 (1.95), Anmol (1.85), Marvi (1.83), Sarsabz (1.82), GP-256 (1.79), Khirman (1.74), Sindh-90 (1.65), Soghat (1.57), Local variety (1.44), Jauhar (1.32), Amber (1.05) and Kiran-95 (0.99).the wheat variety GP-205 show the significantly lower response (0.98). All the tested wheat varieties show the significant difference antibody response against the gliadin (fig. 3).

DISCUSSION

Wheat belongs to six chief food allergens suggest IgE-mediated hypersensitivity reaction manifest like GIT symptoms and respiratory symptoms. While only some wheat proteins causing hypersensitivity reaction to wheat (Peter S *et al.*, 2001).

Sandiford *et al.* (1997) described that gliadins and glutenins are predominantly liable for increase of IgE-dependent reactions. Tanabe *et al.*, (1996b) used Penta peptide QQQPP for rabbit immunizations because this peptide supposed to active allergens surrounded by low molecular protein in wheat flour. Tanabe *et al.*, (2000) described that IgE binding properties of polysaccharides fraction of wheat flour while Watanabe *et al.*, (2001) reported that that IgE binding seen in protein hold carbohydrate moiety.

Tanabe *et al.*, (2001)found the allergenicity of salt soluble protein (globulin) and insoluble protein (gluten) in wheat and concluded that 1-3 patients response against the globulin between 0.15 to >2.0 and response against the gluten in 1-3 patient ranged 0.05 to 0.07 while sensitivity of both fraction varied from 0.16-2.0.

In present study we developed Rabbit antibodies against the different fraction of wheat proteins and our result closed to Tanabe *et al.* (2001).

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