Molecular and Physiochemical Evaluation of Wheat

[1]I. KHAN, [2]M. SHUAIB, [2]Z. ALI, AND [2]A. ZEB*
[1]*Institute of Chemical Sciences, University of Peshawar, Peshawar, Pakistan.*
[2]*Department of Biotechnology, University of Malakand, Chakdara, Pakistan.*

(Received 23rd December 2006, Revised 13th February 2007)

Summary: Different varieties of wheat grains from different ecological regions of Pakistan were evaluated for their molecular and physiochemical characteristics. Thirteen varieties examined included Tatara, Watan, Gandam-711, Bakhtawar-92, Fakhre-Sarhad, Bakkar-01, Ghaznawy, Saleem-2000, Zakht, Gandam-2002, Chudry-97, Inqilab–91 and Wafaq-01. The samples were evaluated for their variation in seed storage protein (gluten) by sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) and for physiochemical characteristics including thousand-grain mass (TGM), moisture contents and minerals contents. On the basis of molecular characteristics, the variety Fakhre-Sarhad was designated as the best variety having higher number of molecular weight-Gluten subunits (HMW-GS), though still requiring improvement in the physiochemical status. On the contrary, Watan and gandam-711, saleem-2000 and Zakht and chudry-97 contained fewer number of HMW-GS as compared to Fakhre-Sarhad variety and were placed in second position. However, these varieties still need improvement in their protein level. The study may prove to be of great future importance in nutritional as well as crop improvement in Pakistan.

Introduction

Wheat is considered as one of the most primitive domesticated crops. Bread wheat plays a major role among the few crop species being widely grown as food sources and was the likely basis for the initiation of agriculture [1]. Presently, global wheat production is concentrated mainly in Australia, Canada, China, European Union, India, Pakistan, Russia, Turkey, Ukraine and the United States, accounting for over 80 % of world's wheat production. Pakistan is the 8th largest wheat producer, contributing to about 3.17 % of the world's wheat production from 3.72 % of the wheat growing areas. Wheat in Pakistan is a leading food grain and occupies a central position in agriculture and its economy [2].

Wheat is supreme among the cereals largely due to the fact that its grain contains protein with unique chemical and physical properties. When ground wheat is mixed with water, the storage proteins bind in such a way that a coherent mass of protein called Gluten is formed. Gluten exhibits plasticity, strength and elasticity, which enable wheat flour to form cohesive dough that can expand to accommodate gas and yet resist stretching to the bursting point. It is mainly because of this unique property of Gluten that wheat is considered the world's most widely consumed source of food. Besides being a rich source of carbohydrates, wheat contains other valuable components such as protein, Minerals (Phosphorus, Magnesium, Iron, Copper, and Zinc) and vitamins like thiamine, riboflavin, niacin and vitamin E. However, wheat proteins are deficient in essential amino acids such as lysine and threonine [3].

Available data on protein improvement show that there is sufficient scope for the development of cereals having high yield combined with high protein contents of superior quality by extensive breeding and screening programs and that deficiencies and imbalances in the amino acid made up of the proteins can be rectified by genetic means [4].

Presently the wheat breeders in Pakistan are paying more attention to evolve new varieties possessing an improved yield potential coupled with superior quality. The physiochemical and quality characteristics of some promising Pakistani wheat varieties have been previously evaluated [5]. Local wheat varieties have been reported to contain protein (10.13- 14.74 %), wet Gluten (23.53 – 38.71 %) and straight grade flour yield (65.78 – 74.40 %). The current study is in line with the

*To whom all correspondence should be addressed.

future importance in nutritional as well as crop improvement [6].

Pakistan has remained a food deficit country since a very long time. The breeding efforts in the past remained focused mainly on increasing the per hectar yield of wheat, thus the potential of grain quality improvement remained unexploited. Production of wheat was geared up to meet the requirements of local market, which is neither quality conscious nor sufficiently diversified to demand exacting standards. Pakistani wheat varieties are grown over a wide agro-climatic range and as such are expected to exhibit yield and quality differences [7]. It is therefore necessary to investigate the molecular and physiochemical characteristics of wheat varieties available for food and nutritional purposes. This study proved important in providing an opportunity to explore the available wheat varieties and to further improve their nutritional quality.

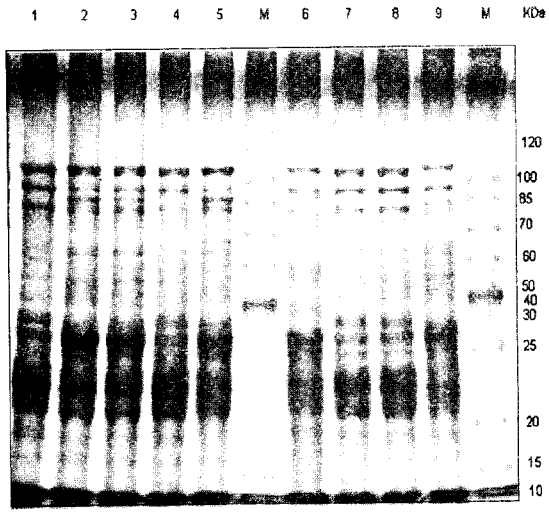
Results and Discussion

Molecular Analysis by SDS-PAGE Electrophoresis

Acrylamide gel electrophoresis in the presence of sodium dodecyl sulphate has become one of the most widely used techniques to separate and characterize proteins. This technique offers two distinct advantages. Polypeptides migrate on SDS gels according to the molecular weight so that molecular weight of polypeptides may be easily and rapidly estimated. At the same time, many insoluble proteins are solubilized by SDS therefore SDS gel electrophoresis has become the technique of choice for resolving mixtures of insoluble proteins, especially membrane proteins. In this study, Gluten subunits of different Pakistani wheat varieties were separated by SDS-PAGE electrophoresis. The picture was taken by gel documentation apparatus with white light illuminator. Data from the picture were analyzed for the types of the protein bands.

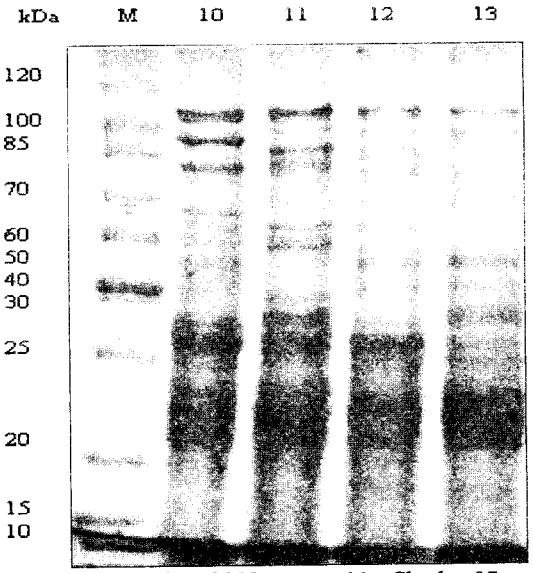
High Molecular Weight Gluten Subunits (HMW-GS)

Data regarding high molecular weight Gluten subunits *(HMW-GS)* are given in Fig. 1 and 2; the data obtained for exact molecular weight of protein as measured from the standard markers are shown in Table- 1. The high molecular weight Gluten subunits of 70, 100 and 120 kDa were absent in all varieties. With the exception of



1= Tatara 2=Watan 3=Gandam 711
4=Bakhtawar-92 5=Fakhre-Sarhad
6=Bakkar-01 7=Ghaznawy 8=Saleem-2000
9= Zakht

Fig. 1. Molecular analysis of wheat varieties (1-9) by SDS-PAGE electrophoresis



10= Gandam-2002 11= Chudry-97
12= Inqilab-91 13=Wafaq-01

Fig. 2. Molecular analysis of wheat varieties (10-13) by SDS-PAGE electrophoresis

Table- 1: Molecular weight analysis of wheat varieties

Protein Type	Molecular Weight (kDa)	Wheat Variety												
		1	2	3	4	5	6	7	8	9	10	11	12	13
HMW-GS	120	0	0	0	0	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0	0	0	0	0	0
	85	0	1	1	0	1	0	0	0	1	0	1	0	0
	70	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	1	1	1	1	1	1	1	1	1	1	1	1	0
LMW-GS	50	1	1	1	1	1	1	1	1	1	1	1	1	1
	40	0	0	0	0	0	0	0	0	0	0	1	0	0
	30	1	1	1	1	1	1	1	1	1	1	1	1	1
	25	1	1	1	1	1	1	1	1	1	1	1	1	1
	20	1	1	1	1	1	1	1	1	1	1	1	1	1
	15	0	0	0	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0	0	0	0

1 = Tatara 2 = Watan 3 = Gandam 711
4 = Bakhtawar-92 5 = Fakhre-Sarhad 6 = Bakkar-01
7 = Ghaznawy 8 = Saleem-2000 9 = Zakht
10 = Gandam-2002 11 = Chudry-97 12 = Inqilab-91
13 = Wafaq-01

Wafaq-01, all the varieties showed a value of 60 kDa HMW-GS. The varieties Watan, Gandam-711, Fakhre-Sarhad, Zakht and Chudry-97 appeared to contain 85 kDa Gluten, while others contained none. It has therefore, been established that HMW-GS of 60 kDa is a more common protein band present in all varieties, with less variation, while only some of the varieties possess 85 kDa, having great variations. SDS-PAGE electrophoresis of seven wheat varieties has been investigated including Inqilab-91 for HMW gliadin. However, their varieties are different than ours but the final result correlated with our results for HMW-proteins [8].

Table- 2 shows molecular weight ranges of different protein bands in different wheat varieties. HMW-GS of 70-120 kDa has similarity with the varieties Tatara, Bakhtawar, Bakkar-01, Ghaznawy, and Gandam-2002 and Inqilab-91 containing 3 major bands, while Watan, Gandam-711, Saleem-2000, Zakht and Chudry-97 contained four similar major bands. Wafaq-01 and Fakhre-Sarhad showed different major protein bands of 2 and 5 in number. With few exceptions, there was no minor band in 70-120 kDa range. In contrast to this, there were no major bands of 50-70 kDa ranges, while minor bands showed a variation similar to major 70-120 kDa ranges.

Table- 2: Molecular weight range of different protein bands of wheat varieties

Protein Type	Molecular Weight Range (kDa)	Band Type	Wheat Variety												
			1	2	3	4	5	6	7	8	9	10	11	12	13
HMW-GS	70-120	Major	3	4	4	3	5	3	3	4	4	3	4	3	2
		Minor	0	0	0	0	0	0	0	0	0	0	0	1	3
	50-70	Major	0	0	0	0	0	0	0	0	0	1	2	0	1
		Minor	4	4	4	4	6	4	4	4	5	5	3	4	3
LMW-GS	25-50	Major	3	3	3	3	3	2	3	3	3	3	4	2	0
		Minor	0	0	0	0	0	0	0	0	0	0	0	0	3
	15-25	Major	3	3	3	3	3	3	3	3	3	4	4	3	3
		Minor	2	3	2	2	2	3	3	2	1	3	4	1	1

1 = Tatara 2 = Watan 3 = Gandam 711
4 = Bakhtawar-92 5 = Fakhre-Sarhad 6 = Bhakkar-01
7=Ghaznawy 8 = Saleem-2000 9 = Zakht
10 = Gandam-2002 11 = Chudry-97 12 = Inqilab-91
13 = Wafaq-01

Low Molecular Weight Gluten Subunits (LMW-GS)

The low molecular weight Gluten subunits of 20, 25, 30, and 50 kDa are present in all varieties, while proteins of 10, 15 and 40 kDa are absent in all the varieties. So, Table- 1 shows the presence of more exact bands as measured from the standard protein markers of low molecular weight as compared to high molecular weight Gluten subunits, which reflect high variations in HMW-GS in all wheat varieties while less variation in LMW-GS.

In LMW-GS, there was less variation in major 25-50 kDa protein ranges, while no minor band appeared in this range. LMW-GS of 15-25 kDa showed less variation than its minor protein bands.

Physiochemical Characteristics

Moisture Contents

Moisture contents were measured in order to know the level of water contents of wheat plant, being essential in terms of productivity as well as for nutritional evaluation. Data regarding moisture contents of different wheat varieties are given in Table-3. The wheat variety Wafaq-01 contained the highest moisture with 0.3547g per 100 grains; while Tatara had 0.3461g moisture. The lowest moisture content was present in Watan with 0.2416 g per 100 grains. Similar results were obtained in our previous study [9].

Thousand Grain Mass

Thousand grain mass (TGM) is an important parameter for the determination of productivity of wheat in a particular region. TGM of different varieties growing in different ecological regions of Pakistan are presented in Table-3. Data represented the high mass of Wafaq-01 and Tatara being 43.602 and 41.484, respectively. Bakkar-01, Inqilab-91 and Bakhtawar-92 have less TGM with a value of 34.025, 33.495 and 31.108, respectively. Jamil and Khan [10] reported a TGM value of 36.29 for Bakhtawar-92, which was 5.182/ g higher than our value of 31.108. The probable reason for this high value could be the freshness of the grains used.

Minerals Composition

The data regarding mineral nutrients of wheat varieties growing at different ecological condition are presented in Table-3. Sodium content of Tatara was 35.9 ppm per 2g of grain sample being placed at first position; Gandam-2002 had 32.8 ppm with second position while the lowest value was recorded in Bakkar-01 with 7.7. A similar trend was observed in sodium contents of these varieties determined by imbibition method [9]. The average potassium content of Zakht variety was 90.6 ppm per 2g of grain sample; Chudry-97 was placed on the second position containing 84.25 ppm. The lowest level of potassium was observed in Ghaznawy being 77.65 ppm.

Phosphorus contents per two grams of sample were determined with the help of UV-visible spectrophotometer in different wheat varieties, which are presented in Table-3. The highest level of phosphorus was present in Watan and Saleem-2000 with a similar value of 0.84 ppm. The lowest value was recorded in Gandam-2002 with a value of 0.24 ppm. Once the basis of

Table- 3: Physiochemical characteristics of wheat varieties

S. No	Wheat Variety	Moisture (g)	TGM (g)	Minerals Contents (ppm)		
				Sodium	Potassium	Phosphorus
1	Tatara	0.3461	41.484	35.9	77.8	0.36
2	Watan	0.2416	35.724	30.6	83.1	0.84
3	Gandam-711	0.2843	39.531	30.95	80.15	0.60
4	Bakhtawar-92	0.2528	31.108	29.4	81.75	0.42
5	Fakhr-E-Sarhad	0.2785	36.860	32.05	81.55	0.53
6	Bhakkar-01	0.2658	34.025	7.7	78.6	0.68
7	Ghaznawy	0.2725	35.133	31.25	77.65	0.44
8	Saleem-2000	0.2822	36.071	31.45	84.15	0.84
9	Zakht	0.2755	35.133	31.05	90.6	0.74
10	Gandam-2002	0.2880	39.297	33.8	83.5	0.24
11	Chudry-97	0.3330	40.210	31.2	84.25	0.68
12	Inqilab -91	0.2580	33.495	31.45	81.15	0.26
13	Wafaq-01	0.3547	43.602	31.25	81.15	0.66

molecular characteristics the variety Fakhre-Sarhad was designated the best variety having a higher number of HMW-GS, while Watan and Gandam-711, Saleem-2000, and Zakht and Chudry-97 contained less number of HMW-GS as compared to Fakhre-Sarhad variety. But this variety needs a little improvement in physiochemical status. However, the other varieties mentioned above, need improvement in their protein levels.

Experimental

Collection of Samples

Wheat grains were collected from different ecological regions of Pakistan. The wheat varieties were stored in labelled glass bottles to ensure safety, in the Department of Biotechnology, University of Malakand, for analysis.

Molecular Analysis by SDS-PAGE Electrophoresis

Wheat grain proteins, especially Gluten subunits were analyzed by using standard SDS-PAGE (Sodium dodecyl sulphate – Polyacrylamide Gel Electrophoresis) method [11-12]. The grains were ground to fine powder and 10 mg was weighed in 1.5ml micro tube, 400 µl protein extraction buffer (Tris-HCl 0.05 M (pH 8), 0.02 % SDS, 30.3 % urea, with 1% (2-mercaptoethanol) being added to each micro tube. The micro tubes were kept overnight at 40 ^{0}C and centrifuged at 13000 rpm for 10 minutes. The supernatant contained extracted protein ready for experimental purposes. The separating gel was prepared by mixing 3ml (1.875 M Tris-HCl pH 8.80), 6.9 ml distilled water, 5 ml (5 % acrylamide), 140 µl (SDS 10 %), 90 µl (APS 5 %) and 14 µl Tetra methyl-ethylendiamine (TEMED). Staking gel was prepared by mixing 1ml (0.6M Tris-HCl, pH 6.8), 7.2 ml distilled water, 1.66 ml (30 % acrylamide), 100 µl (SDS 10 %), 80 µl (APS 5 %), and 9 µl (TEMED). Further, the glass plates were cleaned with 70 % ethanol and fixed by using seal gasket and clips. Separating gel was poured onto the cell and layered with water. After 30 minutes, distilled water was removed and stacking gel was added. Comb was inserted into the stacking gel. Then, the glass cabinet was fixed with electrophoresis apparatus and the electrophoretic filled trays with electrode buffer (25mM Tris, 0.1 % SDS, 192 mM glycine). The wells were then cleaned with running buffer and the sample (12 µl) was loaded along with molecular weight marker (Fermentas, Protein Ladder) 10-120 kDa (5 µl) at the bottom of each well using micropipette connecting the power supply at 80 volts. After electrophoresis, the gel was transferred to the tray containing staining solution and shaken gently for 40 minutes, followed by destaining until the background of gel disappeared. The picture was taken by gel documentation apparatus with white light illuminator. Data from the picture were analyzed for protein bands.

Physiochemical Analysis

Physiochemical parameters like moisture contents, TGM and minerals were determined. TGM and moisture contents were determined according to the method described by Zeb *et al*, [9]. The wet digestion was performed for mineral quantification. The mineral composition (Na^+ and K^+) was determined with the help of flame photometer (Jenway PFP7). The phosphorus content of wheat grain was determined by the reaction of acidified solution of ammonium molybdate containing ascorbic acid and antimony [13]. The phosphate in the plant sample reacted to form an ammonium molydiphophate complex, which is reduced to blue colour solution by ascorbic acid. The amount of light absorbed by the solution was measured at 660 nm with UV-visible spectrophotometer (Shimadzu UV-1700 PharmaSpec). The sample reading was measured from the standard calibration curve.

Acknowledgments

We are grateful for financial assistance provided by Higher Education Commission (HEC) Islamabad, Pakistan, under the Foreign Faculty Research Program. Dr. Taufiq Ahmad, Principal Scientific Officer NIFA, Peshawar, Mr. Waqar Ali, Lecturer Dept. of Biotechnology, University of Malakand and Mr. Hussein Mohd., Lab Assistant were kind enough to help in wheat sample collection and laboratory experiments.

References

1. J. R. Harlan, The early history of wheat. In: *L. T. Evans and W.J. Peacock (eds.) Wheat*

science today and tomorrow. Cambridge University Press, Cambridge UK, pp. **1**, (1981).
2. Govt of Pakistan. Economic Survey of Pakistan, Ministry of Finance, Islamabad. pp. **17**, (2003).
3. R. N. Adsule, and S. S. Kadam, Quality of wheat. In: *D.K. Salunkhe, S. S. Kadam and A. Austin (eds). Quality of wheat and wheat products. Metropolitan Book Co. 1986. New Delhi, India.* pp. **1**, (1986).
4. Z. Akram, Genetic implication of Quality and yield contributing characteristics in Wheat. *PhD thesis. Department of Plant Breeding and Genetics, University of Arid Agriculture, Rawalpindi. Pakistan.* pp: **1**, 7 (2004).
5. F. M. Anjum, W. Butt, M. S. Butt and S. Wahab, *Sarhad. J. Agric.* **14** , 253 (1998).
6. I. Ahmad, F. M. Anjum, and M. S. Butt, *Pak. J. Food. Sci.* **11**, 1 (2001).
7. M. A. Chowdhry, M. Ramzan, K. Alam and I. Khaliq, *J. Agric. Res.* **33** , 71 (1995).
8. M. F. Khan, E. Schumann and W. E. Weber, *Asian. J. Plant. Sci.* **1** , 699 (2002).
9. A. Zeb, Z. Ali, T. Ahmad and A. Abdumanon, *Pak. J. Bio. Sci.* **9** , 1823 (2006).
10. M. Jamil and U. Q. Khan, *Asian. J. Plant. Sci.* **1** , 579 (2002).
11. U. K. Laemmli,. *Nature.* **227**, 680 (1970).
12. P. I. Payne, and K. G. Corfield, L. M. Holt and J. A. Blackman, *J. Sci. Food Agric.*, **32**, 51 (1981).
13. H. D. Chapman, P. F. Pratt, Division of Agriculture Sciences, *University of California, Davis*, p. **162** (1978).