

## GENETIC VARIATION FOR SEED PROTEIN IN BARLEY GERMPLASM

ZAHEER AHMAD AND SHAHEENA YASMIN

*Crop Sciences Institute, National Agricultural Research Centre,  
Islamabad, Pakistan.*

### Abstract

Barley accessions collected from three regions of Pakistan were evaluated for seed protein (%) for two seasons. A wide range of variation (9 to 21%) was found in the germplasm studied during both seasons. Maximum accessions exhibited protein 12.1 ~ 16.0% protein whereas few accessions produced more than 18.0% protein. Correlation between two seasons' data was highly significant indicating the influence of genetic component. Germplasm were classified on the basis of regions. Accessions from Northern areas possessed average higher protein percentage followed by NWFP, Baluchistan and check varieties. Classification on the basis of altitude showed that the accessions collected from 200-800 masl had low protein while those collected from 2601-3000 masl had high protein. This study provides information on important protein sources of germplasm.

### Introduction

For progress in plant breeding, variable genetic material is a prerequisite. The study was undertaken to evaluate genetic variability of barley germplasm based on seed protein and explore variation for future use in selection and breeding programs.

Several researchers have studied protein contents of barley germplasm. He *et al.*, (1989) studied protein content of 6 barley types grown at 19 sites. Protein content was higher at sites in North China than at those in the South. Weltzein & Fischbeck (1990) reported that protein content were higher under favorable growing conditions. Similarly Atanassov *et al.*, (1999) evaluated different traits related to grain quality in 49 naked barley accessions. The effects of climatic factors on different components of quality were studied considering the variation across years. Accessions with high protein were identified for use in breeding programmes.

Sun & Wang (1999) studied a total of 6026 accessions of hull less barley germplasm for genetic diversity of protein. Fan *et al.*, (2002) reported eight barley cultivars that contained more than 12% protein.

### Materials and Methods

One hundred thirty three barley accessions were taken for this study. This collection comprised of 52 accessions from Baluchistan, 28 accessions from North West Frontier Province and 53 accessions from Northern Areas. Two commercial barley cultivars viz. Haider-93 and Sanober-96 were also used as check varieties. Accessions were planted for two consecutive rabi seasons (2006-07 & 2007-08) in an Augmented design and seed harvested were taken for protein contents.

For estimation of seed protein contents, Kjel-Auto Model DTP-3 (Japan) was used. The clean barley grains were reduced to whole meal in Brabender seed grinder. The moisture was determined to report protein values on percent dry basis with moisture meter. Each sample weighing 0.5 g barley flour in duplicate was transferred to clean and

oven dried digestion tubes to which 1 g digestion mixture (4.5g  $K_2SO_4$  + 0.5g  $CuSO_4$ ) had already been placed. Then 5ml concentrated  $H_2SO_4$  was added. It was digested in Acid Mist Scrubber (Model AMS-2) at 400°C for 45 minutes. At completion of digestion, tubes were cooled at room temperature and 15ml deionized distilled water was added (as pre-dilution) and was shaken well before loading sample to Kjel-Auto, Model DTP-3.

The data recorded were averaged and analyzed for basic statistics (mean, range, variance standard error and correlation coefficient) following Steel & Torrie (1980). The data were also analyzed on the basis of regions and altitude.

## Results and Discussion

The data during 2006 ranged from 9.43 to 21.64% with mean value of 15.06% and variance 5.83. Maximum accessions (74) exhibited protein 12.1 ~ 16.0% whereas few accessions produced more than 18.0 percent protein. During 2007 seed protein ranged from 8.59 ~ 21.78% with mean value of 14.35% and had variance 7.34. Few accessions (14) produced more than 18.0 percent protein. Correlation between two seasons' data ( $r=0.42^{**}$ ) was highly significant indicating the influence of genetic component. These landraces represented a valuable genetic resource that could be used to develop new barley cultivars with improved end use quality traits. Dong *et al.*, (2003) evaluated barley accessions for quality characteristics. Data indicated that the quality trait indices of all tested accessions possessed significant variation (Table 1).

**Table 1. Statistics of barley accessions for protein contents.**

Year	Mean $\pm$ S.E	Range	Variance
2006	15.06 $\pm$ 0.21	9.43 ~ 21.64	5.83
2007	14.35 $\pm$ 0.23	8.59 ~ 21.78	7.34

**Table 2. Region-wise statistics of barley accessions for protein contents**

Year	Baluchistan	North areas	NWFP	Checks
2006	9 ~21*	10 ~ 20	10 ~ 21	11 ~ 13
	14 $\pm$ 2.3**	15 $\pm$ 2.2	15 $\pm$ 2.8	12 $\pm$ 1.5
2007	8 ~19	10 ~ 21	9 ~ 19	10 ~ 12
	13 $\pm$ 2.5	15 $\pm$ 2.5	13 $\pm$ 2.2	11 $\pm$ 1.6

\*= Ranges, \*\*= Mean  $\pm$  standard deviation

**Table 3. Altitude-wise statistics of barley accessions for protein contents.**

Year	201-800 (9)	801-1400 (20)	1401-2000 (48)	2001-600 (41)	2601-3000 (15)
2006	9 ~16*	10 ~ 21	9 ~ 21	10 ~ 19	12 ~ 19
	12 $\pm$ 2.1**	14 $\pm$ 2.3	15 $\pm$ 2.5	15 $\pm$ 2.2	16 $\pm$ 2.2
2007	9 ~14	8 ~ 18	10~ 19	10 ~ 21	11 ~ 19
	11 $\pm$ 1.5	12 $\pm$ 2.7	14 $\pm$ 2.3	15 $\pm$ 2.6	15 $\pm$ 2.7

\*= Ranges, \*\*= Mean  $\pm$  standard deviation

**Table 4. Passport data of barley accessions/landraces collected from different parts of Pakistan.**

S. No.	Acc. No.	Province	Lat.	Long.	Alt.
1.	PAK004048	Northern Areas	35°37'	75°63'	2150
2.	PAK004049	"	35°37'	75°63'	2150
3.	PAK004050	"	35°37'	75°63'	2320
4.	PAK004051	"	35°37'	75°63'	2320
5.	PAK004052	"	35°37'	75°63'	2280
6.	PAK004056	"	36°30'	74°64'	2240
7.	PAK004187	Baluchistan	30°21'	67°02'	1650
8.	PAK004188	"	30°21'	67°02'	1550
9.	PAK004194	"	30°74'	66°98	1550
10.	PAK004195	"	30°72'	66°98'	1550
11.	PAK004196	"	30°59'	66°98'	1550
12.	PAK004197	"	30°59'	66°98'	1550
13.	PAK004307	"	30°01'	66°65'	1500
14.	PAK004308	"	30°24'	67°02'	1450
15.	PAK004310	"	30°59'	66°98'	1380
16.	PAK004311	"	30°76'	67°04'	1600
17.	PAK004312	"	29°75'	67°25'	1580
18.	PAK004313	"	30°09'	66°90'	1540
19.	PAK004314	"	28°95'	66°01'	1400
20.	PAK004315	"	29°80'	66°85	1500
21.	PAK004316	"	29°43'	66°58'	1560
22.	PAK004317	"	30°39'	67°37'	1810
23.	PAK004318	"	30°39'	67°55'	2010
24.	PAK004319	"	36°20'	74°15	2120
25.	PAK004320	"	35°85'	75°04'	1306
26.	PAK004322	"	35°42'	75°64'	2080
27.	PAK004323	"	35°42'	75°64'	2060
28.	PAK004324	"	35°42'	75°45'	2140
29.	PAK004325	"	35°37'	75°63'	2520
30.	PAK004326	"	35°37'	75°63'	2500
31.	PAK004327	"	36°05'	74°20'	1380
32.	PAK004328	"	36°31'	74°70'	2140
33.	PAK004329	"	36°40	74°64'	2140
34.	PAK004330	"	36°35'	74°50'	2450
35.	PAK004331	"	36°50'	74°75'	2480
36.	PAK004332	"	36°09'	73°48'	1660
37.	PAK004333	"	36°20'	73°77'	1760
38.	PAK004334	"	36°29'	73°20'	2280
39.	PAK004335	"	36°49'	73°37'	2260
40.	PAK004336	Northern Areas	36°50'	73°37'	2320
41.	PAK004337	"	36°25'	73°37'	1910
42.	PAK004339	"	35°90'	72°49'	1760

Table 4. (Cont'd.).

S. No.	Acc. No.	Province	Lat.	Long.	Alt.
43.	PAK004340	"	36°18'	73°40'	2840
44.	PAK004341	"	36°18'	73°65'	2840
45.	PAK004342	"	36°18'	73°68'	2750
46.	PAK004343	"	36°18'	73°85'	2370
47.	PAK004344	"	36°02'	73°95'	2420
48.	PAK004345	"	36°18'	73°25'	2070
49.	PAK004346	"	35°37'	75°63'	2580
50.	PAK004373	NWFP	35°20'	71°88'	1410
51.	PAK004374	"	36°30'	71°50'	2250
52.	PAK004375	"	36°30'	71°50'	1550
53.	PAK004381	Baluchistan	25°99'	63°52'	370
54.	PAK004382	"	25°99'	63°07'	240
55.	PAK004385	"	30°59'	66°98'	1450
56.	PAK004386	"	29°25'	65°90'	1070
57.	PAK004387	"	30°21'	67°02'	1520
58.	PAK004388	"	29°15'	66°70'	1830
59.	PAK004389	"	29°60'	66°75'	1720
60.	PAK004467	NWFP	34°87'	72°49'	1030
61.	PAK004468	"	34°87'	72°49	1030
62.	PAK004469	"	34°60'	72°70'	800
63.	PAK004471	"	35°02'	72°10'	880
64.	PAK004472	"	34°62'	71°97'	670
65.	PAK004473	"	34°62'	71°97'	670
66.	PAK004474	"	34°50'	71°96'	490
67.	PAK004475	"	34°28'	71°93'	370
68.	PAK004613	"	34°01'	71°95'	290
69.	PAK004620	"	36°02'	71°75'	2740
70.	PAK004621	Baluchistan	29°80'	66°85'	1540
71.	PAK004622	"	29°80'	66°85'	1540
72.	PAK004623	"	29°80'	66°85'	1540
73.	PAK004624	"	28°70'	66°01'	1155
74.	PAK004625	"	28°82'	65°42'	880
75.	PAK004626	"	28°57'	65°42'	800
76.	PAK004627	"	28°05'	65°75'	1350
77.	PAK004628	"	28°57'	65°42'	1400
78.	PAK004629	Baluchistan	28°57'	65°42'	1250
79.	PAK004630	"	28°49'	66°25'	1250
80.	PAK004631	"	26°95'	64°10'	1030
81.	PAK004632	"	26°95'	64°75'	1195
82.	PAK004633	"	28°57'	65°42'	1250
83.	PAK004634	"	27°90'	65°87'	1450
84.	PAK004635	"	27°80'	66°60'	1250
85.	PAK004636	"	27°95'	66°60'	1420
86.	PAK004637	"	28°58'	66°58'	1830
87.	PAK004638	"	29°60'	67°02'	1700
88.	PAK004639	"	30°35'	67°17'	2000

Table 4. (Cont'd.).

S. No.	Acc. No.	Province	Lat.	Long.	Alt.
89.	PAK005106	"	30°37'	67°10'	2015
90.	PAK005107	"	29°10'	66°52'	1835
91.	PAK005108	"	29°09'	66°51'	1765
92.	PAK005109	"	29°80'	66°85'	1820
93.	PAK005110	"	29°03'	66°58'	1885
94.	PAK005111	"	29°15'	66°58'	1905
95.	PAK005114	"	29°56'	66°01'	1225
96.	PAK005115	"	29°90'	66°80'	1720
97.	PAK005116	"	30°35'	67°37'	1785
98.	PAK005121	NWFP	35°20'	71°88'	1255
99.	PAK005122	"	35°25'	71°92'	1415
100.	PAK005123	"	35°35'	71°88'	1440
101.	PAK005125	"	35°90'	71°75'	1850
102.	PAK005127	"	35°90'	71°75'	1545
103.	PAK005128	"	35°85'	71°77'	1710
104.	PAK005129	"	35°95'	71°75'	1750
105.	PAK005132	"	35°90'	71°75'	2225
106.	PAK005133	"	36°25'	72°40'	2830
107.	PAK005134	"	35°90'	71°75'	2950
108.	PAK005135	"	35°90'	71°75'	2950
109.	PAK005136	"	35°95'	71°75'	1765
110.	PAK005137	"	35°94'	71°85'	1990
111.	PAK005142	"	35°90'	71°75'	2060
112.	PAK005151	Northern Areas	36°12'	73°95'	2065
113.	PAK005152	"	36°22'	73°20'	2215
114.	PAK005155	"	36°24'	73°75'	2345
115.	PAK005158	"	36°19'	72°96'	3000
116.	PAK005159	Northern Areas	35°92'	74°29'	1855
117.	PAK005160	"	36°03'	74°29'	2575
118.	PAK005161	"	35°90'	74°15'	2835
119.	PAK005162	"	35°37'	75°73'	2390
120.	PAK005163	"	35°56'	75°62'	2405
121.	PAK005164	"	35°65'	75°62'	2405
122.	PAK005165	"	35°77'	75°62'	2465
123.	PAK005166	"	35°85'	75°62'	2495
124.	PAK005167	"	36°12'	73°95'	2065
125.	PAK005168	"	35°37'	75°80	2395
126.	PAK005169	"	35°37'	76°03'	2500
127.	PAK005170	"	35°62'	76°30'	2505
128.	PAK005172	"	35°32'	76°25'	2640
129.	PAK005173	"	35°14'	76°45'	2685
130.	PAK005174	NWFP	35°01'	76°57'	2705
131.	PAK005175	Northern Areas	35°30'	76°35'	2640
132.	PAK005176	"	35°30'	76°45'	2650
133.	PAK005177	"	35°30'	76°35'	2820

During both years, high level of genetic variation for protein contents could be utilized efficiently for tailoring a new plant variety according to the need of different regions of the country. Sun *et al.*, (1999) studied a total of 6026 accessions of hull less barley germplasm for genetic diversity of spike morphology, some agro economic traits along with protein content and their geographical distribution. They suggested the utilization of potential of advanced germplasm.

Region-wise average protein percentage for two years is exhibited in Table 2 where higher protein percentage was observed in accessions from Northern Areas, followed by NWFP, Baluchistan and check varieties. This suggests that accessions from a particular region should be used to develop barley cultivars having better adaptability by exploiting the regional germplasm. Similarly Gilani & Witcombe (1980) described the distribution of morphological variability of Primitive barley from Northern Pakistan and reported that Pakistani naked barley showed distinct regional variation (Table 2).

Differentiation of accessions according to geographical regions on the basis of agromorphological and biochemical traits is essential not only for its utilization but also to understand the possible regions of diversity (Vavilov, 1951). It has been reported that the accessions from diverse geographical areas of a crop species help to ensure conservation of co-adapted gene complexes (Brown, 1978; Frankel, 1984; Frankel *et al.*, 1995).

On the basis of altitude, it was seen that the accessions collected from 200-800 masl showed low protein. The germplasm collected from 2601-3000 masl had high protein. It was observed that the material under investigation gave high variation for protein contents for most of the collection sites on the basis of altitude. Crossing among selected parents from these identified groups may produce desirable recombinants for further selection Ruiz *et al.*, (1997) studied the relationship between geographical, agromorphological and biochemical parameters in barley landraces. They reported that agromorphological characters like days to heading, maturity and plant height had the highest correlation with the geographical parameters. Association of protein and altitude was also calculated (Tables 3 & 4).

## References

Atanassov, P., A.M. Zaharieva, P. Vendell and P. Monneveux. 1999. Genetic and environmental variation of useful traits in a collection of naked barley II. Quality related traits. *Cereal Res. Commun.*, 27(3): 323-330.

Bothmer, R.Von, N. Jacobsen, C. Baden, R.B. Jorgensen and I. linde Laursen. 1995. An ecogeographical study of the genus *Hordeum*. *Systematic and Ecogeographic studies on Crop Gene pools*, 7: 2nd ed.-IPGRI, Rome.

Brown, A.H.D. 1978. Isozymes, plant population genetic structure and genetic conservation. *Theor. Appl. Genet.*, 52: 145-157.

Dong, Y.C., Y.S. Cao, Y.C. Dong and Y.S.Q. Cao. 2003. Quality characteristics of germplasm resources of food crops and their utilization. *Scientia Agricultura Sinica*, 36(1): 111-114.

Fan, S.J., Y. Li. G.R. Zhang, X.H. Zhu and F. Cao. 2002. Sifting of protein rich, lysine rich barley and analyses of genetic distance. *Acta Agriculturae Shanghai*, 18(1): 29-34.

Frankel, O. 1984. Genetic perspectives of germplasm conservation. pp. 161-170. In: *Genetic manipulation: Impact on man and society*. (Eds.): W. Arber, K. Limensee, W.J. Peacock and P. Starlinger. Cambridge University Press, Cambridge, U.K.

Frankle, O., A.H.D. Brown and J.J. Burdon. 1995. *The conservation of plant biodiversity*. Cambridge University Press, Cambridge, U.K. pp. 249.

Gilani, M.M. and J.R. Witcombe. 1980. The distribution of morphological variability of barley and wheat in a Himalayan center of diversity. *Pak. J. Agric. Res.*, 1(1): 1-8.

Gilani, M.M. and J.R. Witcombe. 1980. The distribution of morphological variability of barley and wheat in a Himalayan center of diversity. *Pak. J. Agric. Res.*, 1(1): 1-8.

He, W., W. Lu and L. Sun. 1989. Effect of environment on protein and starch content of barley grains. *Zuowu Pinzhong Ziyuan*, 1: 25-27. (CAB Abstracts 1987)

Ruiz, M., J.M. Carrillo and F. Varela. 1997. Relationships between some geographical parameters and agro/morphological and biochemical characters in a sample of Spanish landraces of barley (*Hordeum vulgare L.*). *Plant Genetic Resources Newsletter*, 112: 86-89.

Steel, R.G.D. and J.H. Torrie. 1980. *Principles and procedures of statistics*. 2nd Edition, Mc Graw Hill book Co., Inc., New York, USA. pp. 633.

Sun, L.J., W. Lui, J. Zhang, W.X. Zhang, F.Q. Li, L.H. Chen and Y.C. Ren. 1999. Evaluation and utilization of barley germplasm resources of China. *Scientia Agricultura Sinica*, 32(2): 24-31.

Vavilov, N.I. 1997. Five Continents. (Eds.): L.E. Rodin, Semyon Reznik and Paul Stapleton, *International Board for Plant Genetic Resources, Rome Italy*. pp. 1-197.

Weltzein, E. and G. Fischbeck. 1990. Performance variability of local barley landraces in Near Eastern environments. *Plant Breed.*, 104: 58-67.

(Received for publication 3 February 2009)