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Hybridization and Selection for Improving Seed Protein in Barley

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Summary. Hybridization followed by continuous selection of lines of barley from different cross-combinations involving high protein-high lysine genotypes and the agronomically superior strains resulted in breaking the negative correlations between 1000 grain weight and high protein content and high DBC values. The methodology of DBC-Kjeldahl protein adopted in the present study is likely to be useful in identifying high lysine lines. The present study has shown considerable variability with respect to protein content and grain weight and has provided interesting genotypes which can be used in synthesising lines with improved nutritional quality and productivity in barley. The success in breaking the undesirable linkages to factors that impair the endosperm development is due to sufficient genetic variability in the initial breeding material as well as the use of suitable breeding procedures like the full-sib mating in the early segregating generations.

Key words: Barley – Genetic potential – Seed protein – Selection

Introduction

Progress in increasing the genetic potential of barley for higher seed protein has been limited due to the negative correlations existing between protein content and grain yield (Bansal 1974; Doll et al. 1974; Doll and Koie 1978; Hoffman 1950; Munck 1972; Viuf 1972). This correlation could arise from a relative increase in protein content (N × 6.25) in grains and a simultaneous reduction in synthesis of carbohydrate. This is evident from the fact that most of the high-protein and/or high-lysine genotypes of barley have been found to be associated with reduced grain weight (Munck et al. 1969; Bansal 1970; Doll et al. 1974). This is true not only for barley but also for the high lysine genotypes of maize (Mertz et al. 1964) and sorghum (Singh and Axtell 1973). However, strains with a relatively high grain weight and a high protein content, such as B_1, do exist in barley (Bansal et al. 1977; Welsh 1978). In order to exploit the full value of high protein-high lysine genotypes the adverse association between endosperm development and protein quality needs to be broken. With this objective in mind the major emphasis of the present study was upon bringing about changes in the negative relationship between grain weight and protein content and quality in barley.

Materials and Methods

The experimental material was comprised of 344 entries in F_4-F_7 and 44 entries in F_5-F_8 selected from different single and double cross combinations of Barley (Hordeum vulgare, 2n = 14). Nine high protein and/or high lysine genotypes (Hiproly, Notch-1, Notch-2, Riso 7, Riso 8, Riso 29, Riso 56, Riso 1508 and Sv 73608 (Hiproly × Mona[5]), and eight to ten cultivars/strains (DL 70, 'Himani', 'Jyoti', NP 113, BM 21, RS 6, 'Ratna' and R-16) were included for comparison with the derivatives from the crosses. The crosses were made in different combinations among the high protein-high lysine donors Hiproly, Notch-1, Notch-2, B_1 (derivative from Hiproly) and the dwarf mutants BM 18-A and BM 21 as well as the cultivar 'Jyoti'. Selections in the segregating generations, including biparental progenies corresponding to F_3, F_4 through to F_7, were based on good plant type, plump grains, protein percentage and DBC (Dye Binding Capacity) values. Forty-four lines having higher values of protein content and 1000-grain weight (TGW) above the population means were selected and their progenies harvested and analysed in 1979. One gram of whole grain meal from each of the derivatives, high protein-high lysine (HP-HL) genotypes and the cultivars, ground in Udy Cyclotec Mill, was used for protein estimations (N × 6.25) by the macrokjeldahl method on the Tecator Kjeltec System II. DBC (mg dye bound/g sample) analyses were done on the same ground samples using the Udy Protein Analyzer according to Mossberg (1969). Standard

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statistical procedures were followed for the analysis of data. The analyses were conducted on a Borroughs-4700 computer at the Indian Agricultural Statistics Research Institute, New Delhi.

Results

Strainwise averages along with their standard errors, coefficients of variation and the ranges for protein per cent, DBC, TGW and milligram protein per grain are given in Table 1. Mean protein per cent in the selected lines was significantly higher than that in the cultivars and the increase was of the order of about 13%. It was, however, of the same order as that in HP-HL genotypes. The coefficients of variation for protein content in HP-HL genotypes (18.43) and the derivatives (14.61) were higher than that in the cultivars (7.02), indicating scope for further selection in the derivatives.

The selected derivatives and HP-HL lines had significantly higher DBC values than the cultivars. During 1979 the derivatives were equally good as the HP-HL lines for DBC. During 1978, the derivatives (344) were only marginally superior to the cultivars but had a higher range of DBC values comparable to the HP-HL genotypes. Therefore, the increase in the DBC values of derivatives could be ascribed to the effectiveness of selection.

Changes with respect to the TGW of cultivars and the derivatives were not significant though the increase was of the order of 12% in the derivatives. The derived progenies also showed a significant increase in grain weight over the HP-HL lines and this increase was to the extent of about 27%. There was no difference in variation for TGW in HP-HL genotypes and the selections.

Mean protein per grain for the derivatives was significantly higher than that of HP-HL genotypes and the cultivars. The respective increases were about 21% and 26%. The variation in protein per grain was highest in the derivatives (Table 1). The increase in TGW and mean protein per grain in the derivatives over the other two categories was an indication that increased seed size was not at the expense of protein content.

Response to Selection

A positive response to selection in protein content and TGW was obtained and the increase in protein per cent and grain weight in the selected population over the base population was of the order of about 18% and 9%, respectively. Correlated characters, DBC and protein content per grain, also showed a positive response to selection and the increase for DBC and protein per grain were about 8% and 28%, respectively (Table 1).

Compared to the base population, the coefficient of variation was drastically reduced in the progenies of the selected population though there was not much change in these characters in the cultivars and HP-HL genotypes for two years. This indicates that homogeneity for protein and yield characteristics was achieved in the advanced generation (F_5-F_8) material of barley over the base popu-

Table 1. Means and coefficient of variations for protein and yield characteristics in different strains of barley

Traits / Strains	Item	Protein (%)		DBC (mg dye bound/g sample)		1000 grain weight (g)		Protein per grain (mg)	
		1978	1979	1978	1979	1978	1979	1978	1979
Cultivars	No.	8	10	8	10	8	10	8	10
	Mean	12.29	11.86	34.88	33.93	39.99	42.42	4.91	5.00
	S.E.	0.30	0.34	0.89	0.63	2.88	1.67	0.37	0.16
	C.V.	7.02	9.15	7.24	5.84	20.34	12.43	21.11	10.38
	Range	10.6-13.1	11.2-13.5	30.30-37.65	30.20-36.55	29.6-48.8	33.5-49.6	3.50-6.10	4.19-5.71
High protein-high lysine genotypes	No.	9	10	9	10	9	10	9	10
	Mean	14.78	14.39	44.33	41.53	35.18	36.12	5.12	5.15
	S.E.	0.91	0.73	1.34	1.42	1.90	2.17	0.27	0.31
	C.V.	18.43	16.10	9.10	10.82	16.23	19.00	16.03	19.26
	Range	11.7-20.8	11.4-20.4	37.25-52.05	34.60-51.20	27.8-43.6	28.4-50.0	4.02-6.84	4.06-7.35
Cross-derivatives	No.	344[a]	44[b]	344[a]	44[b]	344[a]	44[b]	344[a]	44[b]
	Mean	13.84	16.37	37.14	40.29	44.86	48.87	6.22	7.99
	S.E.	0.11	0.17	0.19	0.29	0.41	0.78	0.08	0.14
	C.V.	14.61	6.80	9.47	4.80	16.83	10.55	23.83	11.43
	Range	8.1-19.3	14.0-19.0	27.90-47.00	37.00-44.70	29.7-65.5	38.8-61.9	3.65-11.41	6.48-9.78

[a] Base population (F_4-F_7)
[b] Progenies of the selected lines (F_5-F_8)

lation. Continuous selection by the pedigree method resulted in the simultaneous increase in the protein content and TGW. The magnitude of increase in protein per grain was to the extent of 55-60% more than in the cultivars and HP-HL lines (Table 1).

Correlation Studies

DBC and protein per cent showed highly significant positive correlations in the three categories of plants (Table 2). This is also evident from the graph plotted between DBC-Kjeldahl protein (Fig. 1a, b). Different regression models were fitted, DBC (Y) as dependent variable and protein % (X) as independent variable, for the entire population of cultivars, HP-HL lines and cross-derivatives for the years 1978 and 1979 respectively in order to study the shape of the curve connecting Y and X. It was observed that the linear model was the best fit as it accounted for more than 90% of the total variation. The equations for the years 1978 and 1979 are $Y = 16.4414 + 1.5064\ x$ and $Y = 19.0058 + 1.3339\ x$ respectively. Taking these equations as normal lines (0% DBC), lines having + 5% and + 10% DBC values were drawn in Figures 1a and 1b.

The association between DBC and protein content was similar in the derivatives as well as in HP-HL genotypes and the cultivars. Thus, the proportion of basic amino acids, including lysine, as measured by DBC, in relation to protein, was not substantially altered in the derived lines in both the years. However, the large negative association between DBC and TGW observed in the cultivars and HP-HL lines was broken in the derivatives where the correlation in the selected lines was not significantly different from zero (Table 2, Fig. 2a, b). This made it possible to obtain a number of combinations of DBC and TGW. Similarly, the insignificant correlation between DBC and protein/grain in the cultivars and HP-HL lines was improved by a large significant positive correlation in the derivatives. In addition, the negative association between protein % and TGW in the cultivars and HP-HL lines was completely eliminated to near zero in the derived progenies (Fig. 3a, b). Thus, the association between seed size, protein content and DBC was improved in the derivatives (Table 2).

Effect of Kernel-Rows

Two-rowed and six-rowed selections were analysed separately for means and variability of protein content and yield characteristics in order to see the effect of kernel-rows since 2-rowed kernels are normally bigger than 6-rowed kernels. In the F_4-F_7 population (1978) 2-rowed lines had higher means for all the characters studied compared to those in 6-rowed lines which showed higher C.V. (Table 3). The magnitude of increase in 2-rowed selections over 6-rowed selections for protein per cent, DBC, TGW and protein per grain was to the extent of 14.5%, 10%, 21.8% and 40% respectively. These differences between 2-rowed and 6-rowed selections were, however, reduced in the progenies of 44 selected lines (1979). Twenty-five 2-rowed selections showed higher levels of DBC, TGW and protein per kernel by 4%, 13.5% and 14.6% respectively than the 6-rowed selections. Protein percentage reached the same level in both 2-rowed and 6-rowed lines (Table 3).

Correlation studies in 2-rowed and 6-rowed selections revealed that DBC-TGW and protein per cent – TGW were negatively associated in 6-rowed selections (Table 4). The negative correlations became non-significant when the progenies of selected 6-rowed lines were analysed in 1979. There was no correlation between these traits in 2-rowed

Table 2. Simple correlations between protein and yield characteristics

Strains Characteristics	Cultivars		High protein high lysine genotypes		Cross derivatives	
	1978 (8)	1979 (10)	1978 (9)	1979 (10)	1978 (344)[a]	1979 (44)[b]
DBC and protein %	0.8610**	0.8861**	0.9303**	0.8734**	0.8473**	0.8181**
DBC and 1000 grain weight	−0.3760	−0.7559	−0.6360	−0.5775	0.1115	0.0023
DBC and Protein per grain	−0.0944	−0.0835	0.3734	0.1272	0.6344**	0.4728**
Protein % and 1000 grain weight	−0.0340	−0.5966	−0.5496	−0.3606	0.0670	−0.1817
Protein % and protein per grain	0.2617	0.1995	0.5038	0.4301	0.7053**	0.4204**
Protein per grain and 1000 grain weight	0.0541**	0.6632*	0.4279	0.6791*	0.7479**	0.8137**

[a] F_4-F_7
[b] F_5-F_8
* and ** significant at 5% and 1% levels of significance respectively. Figures in parenthesis represent number of observations

lines in 1978. But the correlation between DBC and TGW became significantly negative in the progenies of 2-rowed selections (–0.4353). F_4-F_7 (1978) lines in the categories of both 2-rowed and 6-rowed showed significantly positive correlations between DBC and protein per kernel and between protein per cent and protein per kernel whereas the lines in F_5-F_8 (1979) showed no correlations except between protein per cent and protein per kernel which were significantly positive in 2-rowed selections. In comparison to the pooled data irrespective of kernel-rows (Table 2), the figures on separate analysis of 2- and 6-rowed lines showed a different trend (Table 4).

Discussion

Protein content in cereal grains has been shown to be negatively associated with grain yields. In several laboratories studies are in progress which attempt to combine high yields and increased protein content and quality (Bansal and Bhaskaran 1976; Bhatia et al. 1978; Johnson et al. 1978). Grain weight, being an important yield attribute, has been encountered to be a constraint in breeding barley for improved nutritional quality and productivity (Doll and Koie 1978; Welsh 1978). Until now the progress in breaking the negative correlation between protein content

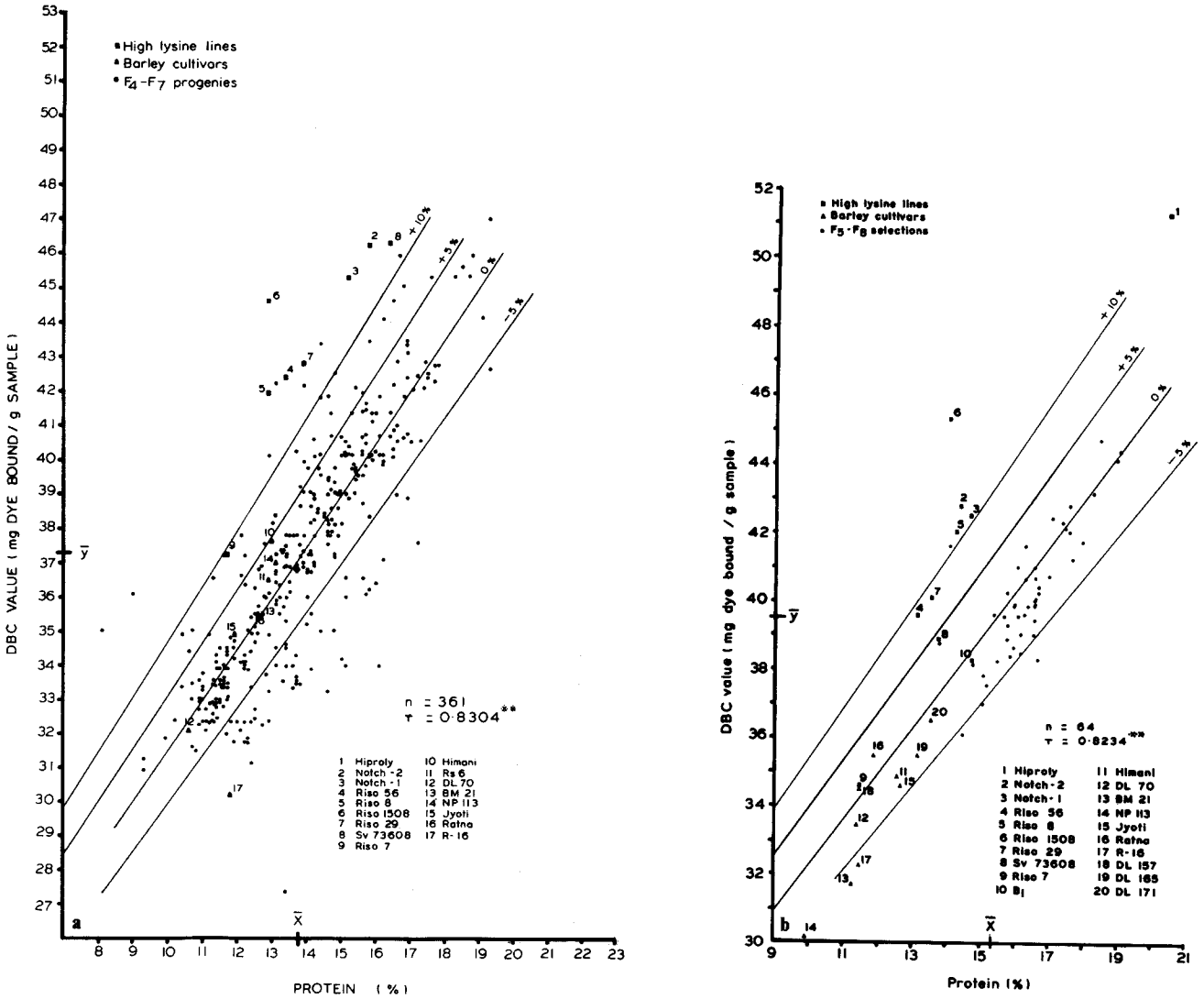

Fig. 1a and b. Relationships between protein % and DBC values in barley cultivars (▲), HP-HL genotypes (■) and cross-derivatives (●), for 1978 (a) and 1979 (b). Lines represent –5%, 0% (Normal) +5% and +10% DBC values

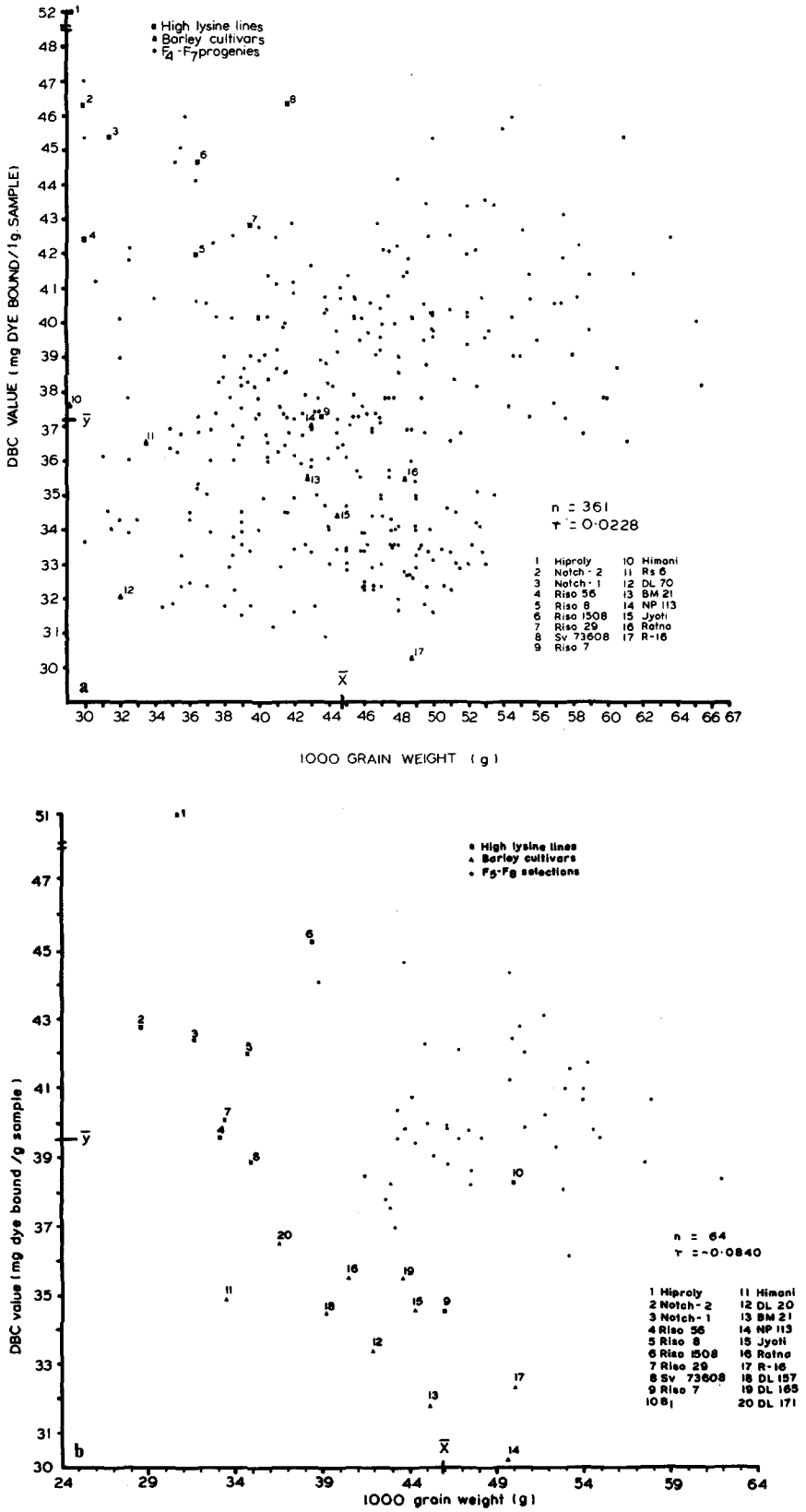

Fig. 2a and b. Relationships between 1000 grain weight and DBC in barley cultivars (▲), HP-HL genotypes (■) and cross-derivatives (●) for 1978 (a) and 1979 (b)

and grain weight was rather limited in barley. In the present study attempts were made to bring about changes in the adverse relationship between high grain weight and high protein content and quality in barley. Selections derived from different cross-combinations in F_4-F_7 progenies formed a population of 344 entries. In this population no correlation was, however, observed between macrokjeldahl protein percentage and TGW (r = 0.0670), or between DBC and TGW (r = 0.1115*) as compared to the negative correlations existing in the other two populations of cultivars and HP-HL lines (Table 2). It has been suggested that some high protein or high-lysine genes may be linked to factors that impair endosperm development (Bhatia and Rabson 1976; Doll and Koie 1978). The present study indicates that it is possible to break such undesirable linkages where the combinations of both fully developed endosperm and protein content could be obtained. This success could be due to the availability of sufficient genetic variability in the initial breeding material as well as the use of suitable breeding procedures such as the

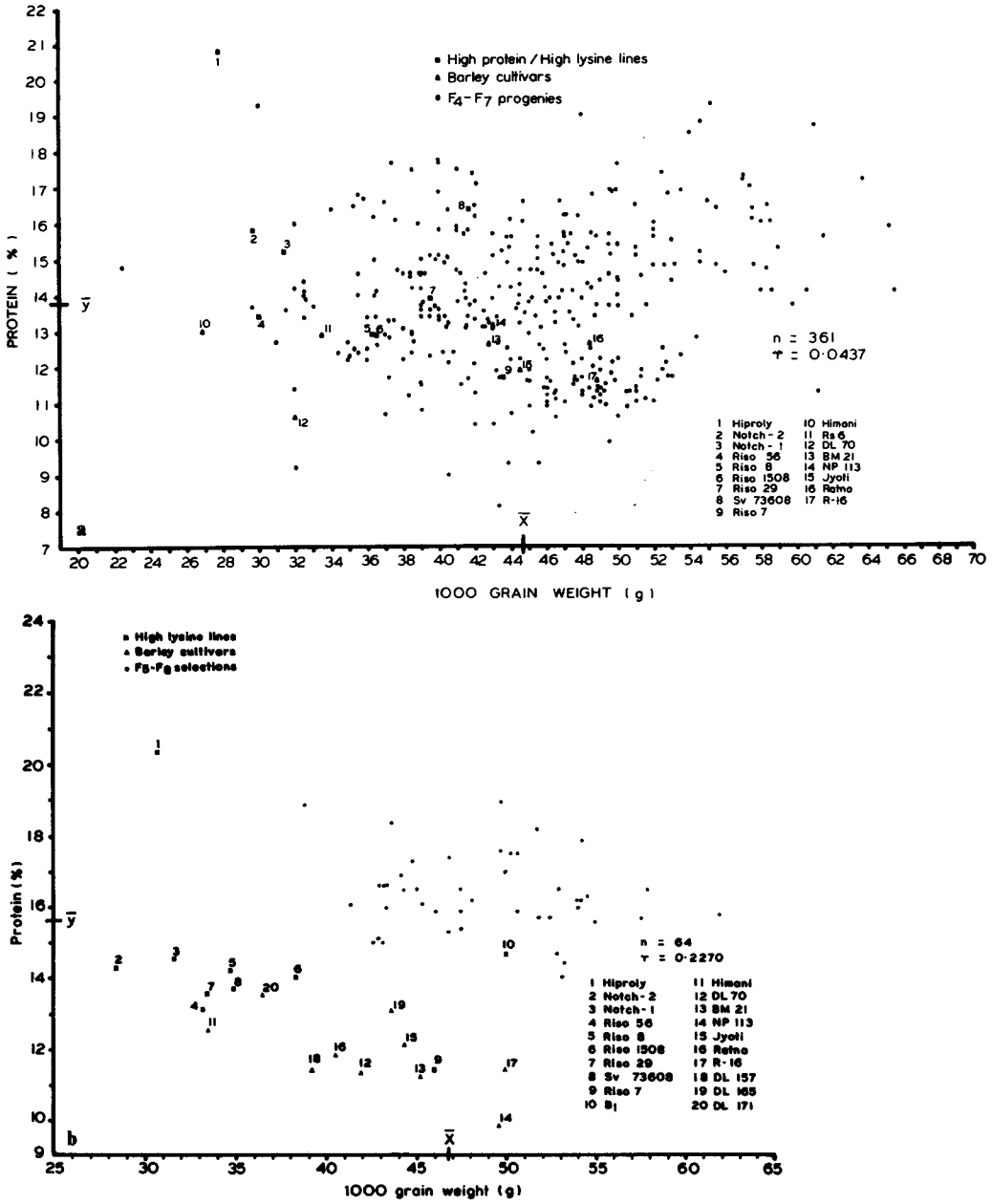

Fig. 3a and b. Relationships between 1000 grain weight and protein % in barley cultivars (▲), HP-HL genotypes (■) and cross-derivatives (●) for 1978 (a) and 1979 (b)

Table 3. Means, standard errors, coefficients of variation and ranges for protein and yield characteristics in 2-rowed and 6-rowed selections

Kernel rows		1978		1979	
Traits	Item	2-rowed (74)	6-rowed (270)	2-rowed (25)	6-rowed (19)
Protein %	Mean	15.36	13.42	16.43	16.28
	S.E.	0.19	0.12	0.25	0.21
	C.V.	10.84	14.23	7.61	5.68
	Range	11.3-19.3	8.1-19.1	14.0-19.0	15.0-18.9
DBC (mg dye bound/g sample)	Mean	40.16	36.31	40.95	39.41
	S.E.	0.33	0.20	0.38	0.37
	C.V.	7.00	8.89	4.69	4.09
	Range	34.00-47.00	31.25-44.10	36.10-44.7	37.00-44.10
1000 kernel weight (g)	Mean	52.19	42.86	51.52	45.38
	S.E.	0.74	0.40	0.73	1.10
	C.V.	12.25	15.27	7.11	10.56
	Range	30.0-65.2	22.5-65.5	43.6-57.8	38.8-61.9
Protein per kernel (mg)	Mean	8.03	5.72	8.45	7.38
	S.E.	0.16	0.06	0.14	0.18
	C.V.	17.40	18.48	8.33	10.86
	Range	4.53-11.41	3.65-9.24	7.16-9.70	6.39-9.78

Figures in parenthesis represent the number of observations

Table 4. Simple correlations between protein and yield characteristics in 2-rowed and 6-rowed selections

Selections	1978		1979	
Traits	2-rowed (74)	6-rowed (270)	2-rowed (25)	6-rowed (19)
DBC – protein %	0.7192**	0.8361**	0.8293**	0.9437**
DBC-1000 grain weight	0.0526	–0.2440**	–0.4353*	–0.1979
DBC-protein per grain	0.4624**	0.5346**	0.3668	0.2673
Protein %-1000 grain weight	0.1335	–0.2839**	–0.3684	–0.1809
Protein %-Protein per grain	0.7026**	0.6470**	0.5864**	0.3138
Protein per grain-1000 grain weight	0.7917**	0.5405**	0.5353**	0.8757**

Figures in parenthesis represent the number of observations
* and ** significant at 5% and 1% levels respectively

full-sib mating in the early segregating generations (Bansal and Bhaskaran 1976).

Continuous selection above the population mean yielded positive response to selection for high grain weight, protein percentage, DBC values and protein per grain. Selection in advanced generations resulted in several lines characterized by different protein levels in comparison to the standard varieties. Interestingly, the increase in TGW in the selected population (1979) in comparison to the cultivars was only 15% while the increase in protein level was to the extent of 38%. This was also clear from milligram protein per grain which was higher in the selected lines. Thus, it is obvious that increase in grain weight need not have any adverse effect on protein accumulation. Increase in protein per grain by about 55-60% (Table 1) is suggestive of the development of lines having better protein synthesis along with starch synthesis. Our results showed highly significant positive correlations between protein per grain and protein per cent as well as protein per grain and TGW (Tables 2, 4). This study also supports the view that selection based on protein content per seed will help to avoid selecting genotypes that have reduced starch synthesis (Nelson 1969; Singhal et al. 1978). This parameter seems to be less influenced by environmental factors than the percentage of protein (Favret et al 1970).

A detailed knowledge of carbohydrate synthesis in re-

lation to protein synthesising capacity of recombinants will go a long way in understanding the processes leading to higher protein production in barley. It will be worthwhile to extend the work on starch characteristics in the derived lines from the crosses involving high-protein and high-lysine donors and the agronomically superior strains since it was found that the starch morphology and its size have characteristic differences in the known genetic sources of high protein/high lysine in barley.

The recombinants included 2-rowed, 6-rowed, hulled and hull-less types. In 1978 2-rowed selections exhibited a higher protein content than the 6-rowed selections but this trend was altered by further selection in 1979 when 2-rowed and 6-rowed lines showed the same level of protein content in grains (Table 3) though the differences between 2-rowed and 6-rowed lines for 1000 grain weight were about 13%. This is in contrast to the observations made by Barabacki (1947) that 2-rowed contained more nitrogen than 6-rowed hybrids. The present study shows that trends in protein content in relation to kernel-rows in barley can be changed by inter-crossing and selection.

Since Acilan Orange G dye binds specifically to basic amino acids, the DBC method as related to Kjeldahl protein has been used extensively in barley to identify high lysine genotypes (Mossberg 1969; Hagberg & Karlsson 1969; Doll et al. 1974). It has been observed that high DBC value can also be due to increase in protein content. This methodology of DBC-KP adopted in the present study showed that different high lysine genotypes identified in barley fall above 10% higher DBC value than the lines showing normal lysine content (Figs. 1a, b) irrespective of their protein contents. Therefore, it is not unreasonable to assume that all the lines falling above the 10% higher DBC value may be high lysine lines. Confirmation of this relationship on the basis of amino acid analyses is underway.

As a result of our endeavours there was a considerable variability with respect to protein and grain weight which suggests that hybridization and selection utilizing genetic sources of high protein-high lysine, may provide interesting genotypes which can be used in synthesizing new hybrids with improved nutritional quality and productivity in barley.

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