Meddling Wheat Germplasm to Augment Grain Protein Content and Grain Yield

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Association of grain protein content with grain yield components was critically examined in (*Triticum aestivum* L.) to facilitate development of genetic resources blended with yield and protein. Investigations carried out for four years on 400-450 line/year, revealed that grain weight plays a defining role in augmenting protein content with grain yield. Even though the extent of variability matched in the four populations, negative association between grain size and protein content was not registered when population mean improved and the trend remained unchanged even when shrivelled or poorly developed grains were removed. Negative association between grain size and protein content also weakened when very small grains were separated. A population with increased grain weight enhanced protein yield and it made selection easier. Population with improved grain weight provided an equal opportunity of selecting genotypes of improved grain yield and grain protein with no deficit on genetic variability. The study demonstrated that grain weight can be articulated to combine yield and grain protein content more efficiently.

Key Words: Bread wheat, Diversity, Grain protein content, Grain weight, Protein yield, Selection efficiency, Yield components

Introduction

The wheat research in India is getting increasingly endowed to grain quality. Genetic improvement in grain protein content of bread wheat (*Triticum aestivum* L.) is crucial for nutritional security and for also improving bread-making quality and the flour yield. A strong inverse relationship between grain protein content and grain yield has been a problem in blending these two traits in high proportion (O'-Brien and Ronalds, 1984; Levy and Feldman, 1987, 1989; Beiquan *et al.*, 1994; Fabrizius *et al*., 1997; Asseng and Milroy, 2006). Any morphological trait useful in removing negative association between yield and protein content shall be immensely useful in selecting genotypes blended with protein and yield but such a possibility has been negated by Martre *et al.* (2007). Since yield and protein are influenced by grain weight in disparate directions, any strategy to augment grain yield and protein content in wheat has to ensure a delicate balance with grain weight. A critical association between grain weight and grain protein content has been articulated for enhancing grain protein content in the background of high yield.

Materials and Methods

Germplasm consisting of entries from the national and international programmes was examined for four successive crop seasons during 2004-07. The material under study was developed as the germplasm-enrichment efforts focussed on quality improvement. Each year some new entries were added to the genetic resource retained from the previous year. The selections were exercised basically to discard the entries which exhibited late flowering, poor development, tendency to lodge, high susceptibility to rust diseases and very low grain protein content. The resource saved each year was augmented with new accessions next year and re-examined for the traits under study. The testing material in the final year therefore, had a good proportion of genotypes superior in 1000 grain weight (TGW) and grain protein content (GPC). Planting was done under irrigated conditions in the middle of November in plot size of 1.2 m^2 *i.e.* (two rows of 2m row length spaced at 30 cm) with recommended agronomic practices, under Augmented Block Design to adjust components under study as per block variations. GPC recorded on Infra-Tec 1255 instrument was converted to 14% grain moisture. The instrument based on infra-red transmission was calibrated with universal software updated for Indian wheat and had been used earlier in several thousand Indian wheat samples for estimation of GPC. Observations were recorded on grain yield, plant height, days to heading, maturity period and TGW. Simple correlations were derived between protein and targeted yield components. Based on average grain weight, the material was further

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assorted into two groups, and the pattern of variability and yield protein relationship was compared each year as suggested by Cochran and Cox (1957).

Results and Discussion

Morphological Traits and Protein Content

The germplasm evaluated during experimental period (four years) exhibited wide range of variability, for all the traits (Table 1). During this period, the coefficient of variability ranged from 6.58 to 7.26% for GPC and 21.5 to 28.6% for grain yield. Early heading and early ripening was noted in the first year material (Set I). Germplasm evaluated in the second year *i.e.* Set II exhibited more diversity and higher GPC but the seeds were small. This difference could be attributed to i) the suppressing growth conditions in the second crop year as expressed through lower yield resulting from less number of grains and poor grain weight which resulted in elevated GPC, and ii) the new accessions augmented in the Set II. Material in the third year (Set III) appeared more uniform and improved for important field characteristics, particularly the phenological attributes, plant height and TGW but protein content was low due to more number of grains. The final year material (Set IV) was characteristically different with high GPC as well as TGW. Selection during past three years led to higher TGW and GPC as negative linkage was broken between these two traits and +ve alleles for both got accumulated in the final year material. In the first two years of Set I and II, the grain yield was negatively correlated with grain weight. On the contrary, when grain weight improved as in Set III and IV, there was no association between grain yield and grain weight. In those populations, it was the grain number which regulated the grain yield with negative correlation between them. As per convention, a negative association between yield and protein was too obvious in all the years. As reported earlier by Beiquan *et al.* (1994), some of the phenological traits also registered association with GPC in some of the years but it appeared totally germplasm specific. Amongst all the traits under study, there was some consistency between grain weight and protein content. A strong negative correlation between GPC and TGW was observed in Set I and II, the populations possessing low TGW. However in improved grain weight populations of Set III and Set IV, such an association was not observed. Strong inverse relationship between TGW and GPC has been reported by Nagarajan *et al.* (2007) and absence of such an association is also not so uncommon (Levy and Feldman, 1987).

It can be concluded that negative association between TGW and GPC was not due to shrivelled grains alone because the trend remained negative in Set I (N: 259, r: -0.28**) and Set II (N: 372, r: -0.35**) and unrelated in Set III (N: 479, r: -0.08) and Set IV (N: 369, r: 0.10) even after delimiting the four populations from late heading and long duration. Multiple regression analysis revealed that indices based upon the morphological traits alone could not be devised to aid selection of high protein genotypes. Though the regression coefficient was significant in each population, the six characters under study could explain very little variability for GPC as the R^2 value varied from 0.08 (Set IV) to 0.23 (Set I). Also, there was no consistency regarding components contributing significantly to high protein in the individual

Table 1. Variability in lines for yield contributing traits and their association with protein content

Characters	SET I 2004 (373 lines)			SET II 2005 (450 lines)			SET III 2006 (523 lines)			SET IV 2007 (428 lines)		
	Mean	CV	r value	Mean	CV	r value	Mean	CV	r value	Mean	CV	r value
GPC (%)	12.6	6.58	1.00	13.1	7.26	1.00	12.5	7.04	1.00	12.9	6.41	1.00
GY (g/m^2)	435.0	21.50	-0.18**	407.0	28.6	-0.24**	487.0	23.00	-0.29**	402.0	25.4	-0.21**
TGW (g)	33.5	14.10	-0.39**	32.1	15.5	-0.36**	36.9	12.50	-0.07	39.5	13.3	0.08
GPM (‘000)	13.1	21.20	0.04	12.8	28.4	-0.06	13.3	23.40	-0.24**	10.3	25.6	-0.23**
PH (cm)	104.0	9.50	0.18**	107.0	9.92	0.07	102.0	8.68	0.18**	99.0	10.0	0.04
HD (days)	90.0	6.07	0.28**	93.0	8.80	0.13**	95.0	4.06	0.03	93.0	6.38	-0.03
GGP (days)	43.0	10.90	-0.27**	52.0	12.6	-0.04	45.0	6.73	-0.07	48.0	11.5	-0.02

CV = Coefficient of variability (%), r value = Correlation coefficient with grain protein content,
** = Significant at P 0.01, GY = Grain yield, GPM = Grains per m^2, PH = Plant height, HD = Heading days,
GGP = Grain growth period

populations. It was TGW, height and grain growth period (GGP) in Set I, heading and GGP in Set II, height in Set III, and TGW and GGP in Set III.

Grain Weight and Protein Relationship

Balance between grain weight and grain yield has always been crucial in wheat. Since yield in wheat is derived through number of grains per unit area and grain weight, negative relationship between TGW and GPC is bound to hamper breeding efforts aimed to blend high protein content and high grain yield. An understanding of the magnitude and direction of the association between TGW and GPC is, therefore, very crucial in breeding for high protein content without compromising TGW. It was observed that instead of linear, the polynomial trend fitted better between these two important grain traits (Fig. 1). R^2 value improved in all the four populations when polynomial trend line of the order 2 was plotted. It is evident from the present study that in the populations with low TGW, negative association between these traits existed only in the lot where TGW fell below 32-33g. Therefore, negative association between grain weight and protein content should not always be taken as disincentive in quality improvement efforts.

Selection for Protein Yield

Grain yield and grain protein concentration are two important parameters in breeding for high protein content in wheat. Independent segregation of the genes controlling grain yield and protein concentration had been reported to suggest simultaneous selection for the two components (O'-Brien and Ronalds, 1986, Fabrizius *et al.,* 1997). It has also been suggested that overemphasis on selection for high protein content can reduce yield in the following generation (O'-Brien and Ronalds, 1986). Alternatively, selection for protein yield per unit area has also been in practice since long (Bhatia, 1975; McNeal *et al.,* 1982). In the present study, the path analysis was done to ascertain importance of component traits. Since protein yield is a function of GPC and yield, high direct effects of GPC and grain yield were quite obvious in each population. However, importance of grain weight

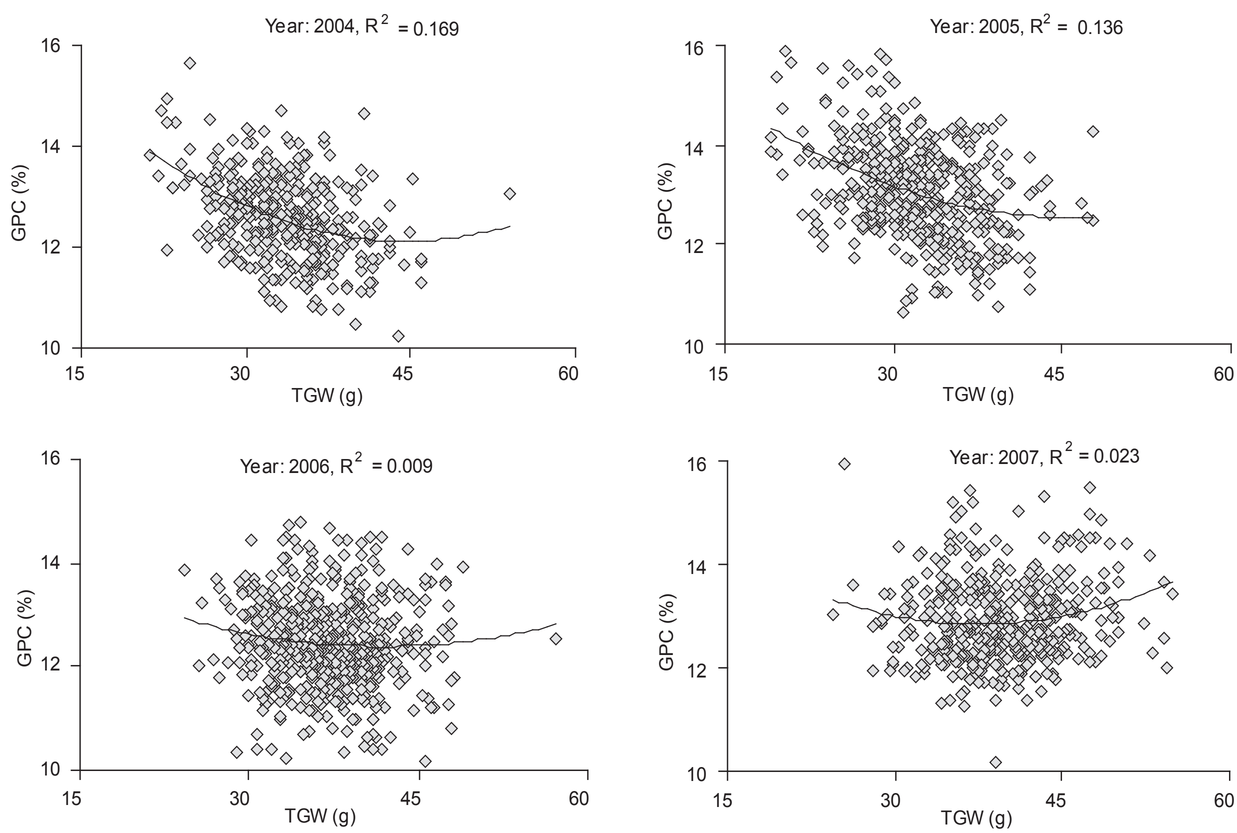

Fig. 1: Relationship between grain weight and protein content

and number of grains per unit area was also noticeable in Set I and Set II, the two populations with poor grain size. In Set III and Set IV, the grain number and grain size had negligible direct contribution. It implied that if populations are improvised for grain size, protein yield can be augmented with ease by selection based upon GPC and grain yield.

Table 2. Direct effects on protein yield in wheat

Components	2004	2005	2006	2007
Grain protein content	0.296	0.256	0.319	0.246
Grain weight	0.077	0.080	0.039	0.001
Grains/ m^2	0.117	0.142	0.072	0.011
Grain yield	0.893	0.883	0.971	1.010
Plant height	-0.000	0.001	-0.003	-0.006
Heading days	-0.005	0.014	-0.003	-0.009
Grain ripening duration	-0.000	0.017	-0.001	-0.005
Residual	0.004	0.006	0.005	0.004

Multiple regression analysis further confirmed the utility of the material improvised for TGW. Besides GPC and yield, grain number and grain weight were again significant constituents of protein yield when populations were low in TGW, whereas a population with improved TGW (Set IV) derived protein yield from GPC and grain yield only.

Table 3. Regression analysis for protein yield

Regression Statistics	2004	2005	2006	2007
R^2 value	0.996*	0.994*	0.995*	0.996*
Intercept	-60.320	-66.030	-62.940	-45.970
Regression coefficient				
Grain protein content (%)	4.241*	4.053*	4.839*	3.804*
Grain yield (g/m^2)	0.113*	0.114*	0.116*	0.127*
1000 Grain weight	0.192*	0.238*	0.112*	0.004
Grains/ m^2 ('000')	0.500*	0.583*	0.304*	0.061
Plant height (cm)	-0.001	0.001	-0.005	-0.007
Heading days	-0.009	0.025	-0.009	-0.020
Grain growth period (days)	0.001	0.037	-0.004	-0.013

*Significant at P 0.001

Diversity in Grain Weigh-improvised Populations

Importance of grain weight in augmenting protein content was further investigated by assorting the study material of each year into two groups and the average TGW of that season was used as the dividing line. The group with improved TGW in each population was labelled 'Elite' and the extent of variability was compared with that of the total population (Set). It was observed that even after sieving the population, the elite populations registered no loss in the extent of variability for both GPC as well as protein yield (Table 4). Therefore, equal opportunity persisted while selecting the right type of genotype for GPC and protein yield. The elite groups had an inbuilt advantage with respect to protein yield, over the years.

Step-down multiple regression analysis further suggested that selection for protein yield was easier in the grain weight improvised populations (elite groups) as it was based solely on two traits i.e. yield and GPC. Unlike the parental populations, characters like grain weight and number of grains per unit area (represented by number of spikes, spikelets and spike length) had marginal influence in the elite groups (Table 5).

Conclusions

High grain weight, an important yield component, is regarded as a bottleneck in enhancing grain protein content and this adverse relation can not just be attributed to shrivelled grains. A strong correlation in positive direction with grain yield and in negative direction with that of GPC makes TGW a very tricky characteristic in wheat breeding. However, it can be made to serve as stratagem to improvise the selection methodology required for selecting genotypes with high GPC or protein yield. It can be articulated by sieving the working populations against low TGW i.e. ≤ 30-35g (depending upon the crop season). A large and diverse population might pose problems in selection but such an assortment downsizes the base population with no bearing on variability for protein content and protein yield and no fear of inverse relationship between TGW and GPC. In this improvised population, selection gets easier and more effective, especially in case of protein yield. Route to protein yield is also different in such elite populations. Grain weight and grain number per unit area accumulating from tillering, spike length and number of spikelets becomes redundant and unlike original populations, protein yield just becomes direct function of yield and protein content. Such a selection technique therefore may pave way for harnessing enhanced grain protein content in the background of high yield.

Table 4. Extent of variability for protein content and protein yield in improvised populations

Group	2003-04		2004-05		2005-06		2006-07	
	Total	Elite	Total	Elite	Total	Elite	Total	Elite
Lines	373	189	450	230	523	257	428	211
TGW	21.2-54.0	33.6-54.0	19.2-47.6	32.1-47.6	24.3-57.1	37.0-57.1	24.4-54.8	39.1-54.8
Grain protein content (%)								
Range	10.2-15.6	10.2-14.6	10.6-15.9	10.8-14.5	10.2-14.8	10.2-14.7	10.1-16.1	11.4-16.1
CV (%)	6.58	6.35	7.26	6.75	7.04	7.24	6.41	6.25
Mean	**12.6***	12.3	**13.1***	12.8	12.4	12.4	12.9	13.0
Protein yield (g/m^2)								
Range	18.6-97.0	25.6-97.0	19.7-120.5	21.5-111.5	21.2-101.0	21.2-101.0	15.6-87.3	20.5-87.3
CV (%)	21.7	21.9	28.4	26.2	22.1	20.9	24.8	23.8
Mean	54.6	56.3	53.0	55.5	60.4	**63.1***	51.8	**54.5***

*Significant at P 0.05 (Bold)

Table 5. Step-down regression analysis for protein yield in elite groups

Regression Statistics	Elite group		
	2003-04	2004-05	2005-06
R^2 value	0.997*	0.996*	0.995*
Intercept	-56.130*	-56.480*	-62.210*
Coefficients			
Protein %	4.464*	4.345*	5.009*
Grain yield	0.126*	0.130*	0.124*
1000-grain weight	Nil	Nil	Nil
Grains/m^2	Nil	Nil	Nil
Plant height	Nil	Nil	Nil
Heading days	Nil	Nil	Nil
Maturity period	Nil	Nil	Nil

*Significant at P 0.001

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