Biofortification strategies to increase wheat nutrition and sustaining yield simultaneously

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Abstract

Genetic biofortification is a strategy that uses plant breeding techniques to produce staple food crops with higher micronutrient levels and can be a feasible and cost-effective means of delivering micronutrients to population that may have limited access to diverse diets. The present study reports the development of wheat with high grain protein, yellow pigment and high zinc content in addition to introgression of rust resistance genes to ensure biofortification as well as yield sustainability. A convergent cross for combining gene(s) for high protein, high yellow pigment, high zinc and rust resistance was performed. This included crossing BC_1F_2 introgression lines of cross PBW698/BF22//PBW698 carrying grain zinc QTL introgressed from *Triticum monococcum* with high protein line BWL3560 having *Gpc-B1*. A set of 192 F_3 lines were evaluated for agronomic and quality traits. Molecular marker analysis of F_3 progenies for *Gpc-B1* gene (*Xucw108*) revealed 129 F_3 lines to be homozygous positive. The progenies showed the range of 8.96-13.92% for grain protein content, 25.1-36.3mg/Kg for grain iron concentration, 34.0-54.0 mg/Kg for grain zinc concentration and 2.01-4.57ppm for grain yellow pigment content compared to 9.65%, 26.7 mg/Kg, 42.9 mg/Kg and 3.74ppm respectively in recipient line PBW698. Therefore, this population constituted a useful material for identifying the nutritionally enhanced lines.

Keywords: *Triticum aestivum*, grain iron and zinc, grain protein, *Gpc-B1*, rust resistance genes

Introduction

Wheat represents more than 50 per cent of the daily caloric intake for the majority of world population. The global wheat production achieved a record of 757.92 mt in 2017-18 (www.statista.com). Despite of increment in food production and sufficient staple food intake, near about half of the world's population is suffering from deficiencies of important micronutrients such as iron, zinc and vitamin A. The whole wheat grain contains 20-35 mg/kg of zinc concentration (Cakmak et al. 2004) where most wheat based diets fail to meet even half of Recommended Daily Allowance (RDA) of 10-12mg of zinc (www.icmr.nic.in) required for the human body. A significant percentage of grain zinc concentration is lost with the removal of the aleurone layer and embryo during milling and less than 15 mg/kg were reported to be present in wheat endosperm and in refined flour (Li et al. 2014). Biofortification of wheat with increased micronutrients is a potential mechanism for alleviating micronutrient deficiencies. This holds great opportunity for increasing the nutritional and health status of poor populations in the developing world (Graham and Welch 1996; Underwood 2000; Bouis 2003; Velu et al. 2014; Bouis and Saltzman 2017).

Dietary protein is another critical factor which contributes to human health. Wheat grain in general has 10-11% protein in its grain. Besides the nutritional needs, grain protein content (GPC) constitutes an important economic trait that determines the viscoelastic properties of bread dough of wheat. Therefore, it is an important parameter for deciding quality of wheat cultivar. A major QTL for GPC was first mapped on chromosome arm 6BS from *Triticum dicoccoides* accession FA-15-3 in a population of chromosome specific recombinant substitution lines (Joppa et al. 1997) which was later mapped as a single Mendalian locus named *GPC-B1*, within a 2.7-cM

region (Olmos et al. 2003). Map-based cloning of *Gpc-B1* revealed the gene conferring elevated GPC is a N assimilation control (NAC) transcription factor (TtNAM B1) (Distelfeld et al. 2006) associated not only with increased GPC, but also increased zinc and iron content, leaf senescence, and enhanced N remobilization (Uauy et al. 2006a).Therefore, the well characterised molecularly tagged *Gpc-B1* gene can be effective for transfer of high GPC to superior wheat lines.

Multigenic control along with significant environmental influences for high protein traits (Snape et al. 1993; Kunert et al. 2007) and micronutrients (Trethowan 2007; Joshi et al. 2010) make selections for these more difficult. Generally, a negative correlation between high yield and high protein has been obtained (Simmonds 1995; Blanco et al. 2002; Gonzalez-Hernandez et al. 2004; Brevis and Dubkovsky 2010; Blanco et al. 2012; Wurschum et al. 2016; Rapp et al. 2018). However, this inverse relationship has been debated theoretically. Wheat lines with superior agronomic performance and high grain protein content were also reported in many breeding programs (Singh et al. 2007). Efforts in molecular breeding have been made worldwide for improving GPC and micronutrient content using MAS while selecting against low yield (Kumar et al. 2011; Tabbita et al. 2013). Development of a wheat variety, 'Lillian' (Randhawa et al. 2012) is among few examples of combining high protein and acceptable yield levels.

Wild species, as a potential germplasm resource for genetic improvement of Fe and Zn concentrations have been reported by many workers (Chunneja et al. 2006; Zhao et al. 2009; Xu et al. 2011; Velu et al. 2014; Kumar et al. 2015). The diploid wheat progenitor gene pool comprising *T. monococcum* and *T. boeoticum*, has also been reported to harbour genes for high grain zinc concentration (Singh et al. 2008). At Punjab Agricultural University, Ludhiana, under wheat biofortification program, introgression of genes for high zinc content from *T. monococcum* and *T. boeoticum* to *Triticum durum* (tetraploid wheat) was initiated. This bridging cross was used to transfer the trait to bread wheat since the direct transfer from diploid to hexaploid background is not easy. Grain zinc enhanced tetraploid derivatives then served as donors for improvement in hexaploid wheat varieties. The present study was planned with the objective of combining the above high grain zinc and iron present in the backcross derivatives carrying '*T. monococcum-T. durum*' introgression, with

the high protein line carrying *Gpc-B1* gene within same hexaploid background. With the involvement of durum wheat in the present breeding programme, improvement in a third aspect, *i.e.*, provitamin A carotenoids was also targeted in the breeding populations owing to the introgression of carotenoid specific *Psy1* gene from durum wheat into hexaploid wheat.

Materials and methods

Twenty three BC_1F_2 derivatives of cross PBW698/BF22//PBW698 were crossed with high protein line BWL3560 (carrying *Gpc-B1*gene) in 2014-15 in the Department of Plant Breeding and Genetics at Punjab Agricultural University, Ludhiana. PBW698 used as recurrent parent is a gene pyramided version of PBW343 (carrying rust resistance genes *Yr10*, *Yr15*, *Lr24*, and *Lr28*). BF22 is a tetraploid donor for high grain zinc carrying gene introgression from *T. monococcum* (BC_1F_7 developed from *T. monococcum* W49-27-1 backcrossed to *T. durum* cv Aconchi 89). Forty three F_{1s} were grown at Keylong (Himachal Pradesh) during summer 2015 and F_2 population was generated at Ludhiana during main season 2015-16. In each F_2 population, 200-250 plants were space planted with rows of PBW698 planted at regular intervals for comparison. Ten desirable F_2 populations were shortlisted based on rust resistance and minimum variation within population. Within each selected population, 100 single plant selections were done on the basis of plant type close to PBW698 and rust resistance. A total of 1068 plants were selected and they were phenotypically analysed for grain iron and zinc concentration. Based on grain zinc concentration, 192 genotypes with high zinc (>70 mg/kg) were shortlisted and F_3 lines were sown in 2016-17 main season at Ludhiana for molecular marker analysis and agronomic and quality trait evaluation. The breeding scheme followed to transfer high grain protein to PBW698 is given in Fig. 1.

The field experiment was laid out in a simple lattice design with three replications. Experimental units consisted of 2 rows of 1m length spaced 20 cm apart. Total entries used in this experiment were 196 including 192 F_3 lines from cross [PBW698/BF22//PBW698/3/BWL3560], recipient line PBW 698, grain zinc donor line BF22, *Gpc-B1* carrying line BWL3560 and high yielding variety PBW725 as check. About 10 randomly chosen plants from each F_3 line were bulked and screened with *Xucw 108* (Distelfeld et al. 2006). DNA extraction using CTAB method and molecular
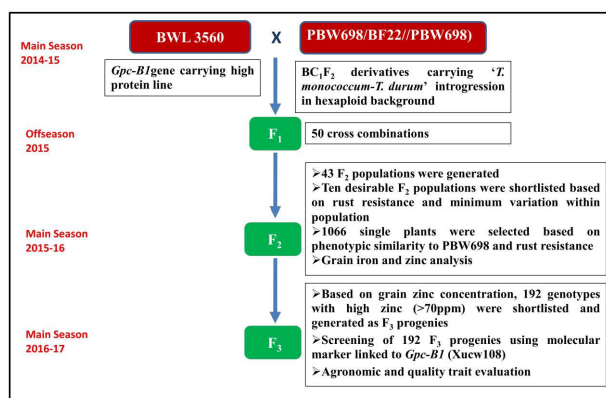
Fig. 1. Breeding scheme followed to transfer high grain protien gene (Gpc-B1) into wheat line PBW698

marker analysis followed using standard procedures (Allen et al. 2006).

Observations were recorded on days to flowering, plant height, days to maturity, tillers per meter row length, spike length, number of spikelets per spike, grains per spike, thousand grain weight, grain yield per plot (g), grain protein content (%), grain zinc concentration (mg/Kg), grain iron concentration (mg/Kg), grain yellow pigment content (ppm) and grain appearance score. Grain appearance score (GAS) was assessed subjectively out of a greatest score of 10 giving due weightage to the grain size (3), shape (2), colour (2) and luster (3). The assessment of stripe rust (*Puccinia striiformis* f.sp. *tritici*) disease severity in the field was done according to Cobb Scale as described by Peterson et al. (1948) for each progeny. It was based on the infection on leaf and reaction type as traces (TS, TR), 5S, 10S, 20S, 40S, 60S and 80S.

The grain protein content was assessed using the whole grain analyzer "Infratec1241" supplied by M/S Foss Analytical AB, Sweden. It utilizes the near infrared light which is transmitted through the grains. The grain samples were scanned in the range of 850 to 1050 nm with a bandwidth of 7 nm and there were 100 data points per scan. The results were displayed as percent protein content along with percent moisture.

Energy dispersive X ray fluorescence (EDXRF) method was used to determine iron and zinc concentrations in wheat grains utilizing an Oxford Instruments X-Supreme 8000 fitted with a 10 place auto-sampler (Paltridge et al. 2012). Estimation conditions were as recommended by the manufacturer for grain zinc and iron analysis in a cellulose matrix. For each sample, total estimation time was 186

seconds (s). Scans were led in sample cups gathered from 21 mm diameter aluminium (Al) cups joined with polypropylene inner cups fixed toward one side with 4 ìm Poly-4 XRF sample film. A circle of 21 mm diameter is scanned by the X-Supreme 8000 with the sample spinner on. All scans in this study were performed in this mode with the scanned area of 346 mm^2.

Yellow pigment content in the wheat wholemeal was estimated using a standard AACC 14-50 calorimetric method (AACC 2000). Four gram wholemeal was weighed and put into a 125ml reagent bottle to which, 20ml water saturated n-butanol was added. Bottle was shaken properly to mix the contents and kept in dark for 16 hours. The extract was obtained after filteration of the contents into standard test tubes. The intensity of colour of extract was read at 440 nm using Spectronic 20$^+$D spectrophotometer and recorded as optical density (O.D.). Yellow pigment content was calculated using the following formula:

$$\text{Yellow pigment content (ppm)} = [(O.D. \times 23.5366) + 0.0105]$$

Results and discussion

Molecular marker analysis of F$_3$ progenies for Gpc-B1 gene

Gpc-B1 was the first gene identified for variation in grain protein content in wheat. *Gpc-B1* gene mapping has advanced through different phases of cytogenetic analysis, RFLP based markers and PCR based markers and was later cloned by Uauy et al. (2006b). Molecular markers *Xuhw89* and *Xucw71* flanked 245-kb physical contig, including *Gpc-B1* (Distelfeld et al. 2006). Presence of this locus showed delayed senescence and increased grain protein content along with increased grain iron and zinc concentrations. The complete sequencing revealed presence of five genes within this region. Molecular markers *Xucw 108*, *Xucw 106, Xucw 87* and *Xucw 97* were developed from this region (Uauy et al. 2006b) that constituted ideal markers for tracing this locus in segregating generations. In the present study, molecular marker *Xucw 108* was used for marker based selection for *Gpc-B1* gene. All the 192 F$_3$ progenies derived from the cross [PBW698/BF22//PBW698/3/BWL3560], were subjected to PCR based marker analysis (Fig. 2) where BWL3560 (carrying *Gpc-B1* gene) was used as positive control and PBW698 as negative control. *Xucw 108* is a codominant marker that amplified 217 bp fragment in positive parent BWL3560 and 190 bp fragment in lines which were negative for this gene.
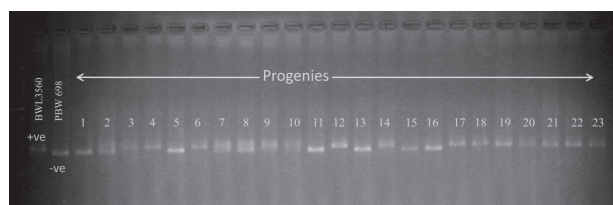
Fig. 2. **Screening of F₃ progenies by xucw 108 for grain protein content**

Out of a total 192 progenies, 129 were homozygous positive for *Gpc-B1* gene, 48 were heterozygous and 15 did not carry this gene. The *Gpc-B1* marker (*Xucw 108*) locus is part of the gene and is expected to actually identify progenies homozygous for gene itself. Therefore the identified progenies mark the gene of interest with high probability.

Evaluation of F₃ progenies for agronomic and quality traits

The F₃ progenies with *Gpc-B1* in the background of PBW698 were evaluated for days to flowering, plant height, days to maturity, number of tillers per plant, number of spikelets per spike, spike length, number of grains per spike, 1000 grain weight, grain yield, grain zinc concentration, grain iron concentration, grain protein content, yellow pigment content and grain score. Significant differences were observed among the genotypes with respect to their mean performance for all the studied traits. Figure 3 shows a set of histograms depicting the frequency distribution of F₃ progenies for grain yield and important quality traits. Wide distribution for most of the studied traits further substantiate the high level of variation observed for these traits. Most of the studied traits including days to flowering, number of grains per spike, thousand grain weight, grain yield and grain yellow pigment content tend to have a normal distribution. Grain protein content and grain appearance score (assessed giving due weightage to the grain size, shape, colour and luster) were highly positively skewed (Fig. 4) along with leptokurtic distribution indicating these traits to be governed by fewer number of genes displaying dominant and dominant based complementary epistasis. For these traits, the gain is slower with mild selection and gain is faster with intensive selection. Days to maturity was moderately skewed along with platykurtic distribution signifying involvement of large number of segregating genes with complementary gene interactions. Grain iron and zinc concentration were also moderately positively skewed (Fig. 4). Plant height and number of tillers were negatively skewed

with leptokurtic pattern representing the presence of duplicate epistasis gene action. The study on distribution properties by third order statistics i.e. coefficients of skewness and kurtosis provides insight about the nature of gene action (Fisher et al. 1932) and number of genes controlling the traits respectively (Robson, 1956).

Thus, a wide variability existed in the present set of 192 F₃ progenies for all the studied traits. This offers an excellent opportunity for identification of nutritionally enhanced lines with good agronomic performance. Three F₃ progenies showed significant improvement over recipient parent PBW698 (208 g/plot) for grain yield while 141 lines were found at par to the recipient parent and only 48 lines were significantly low yielding. The protein content across this set however, varied greatly showing a range of 8.96 (progeny no. 64) to 13.92% (progeny no. 98) with population mean of 10.94% compared to 9.65% in PBW698 and 12.29% in donor parent BWL3560. Out of 192 lines, 101 lines had significant increment in grain protein content relative to recipient parent PBW698 and eighteen lines showed significantly high protein content (>12.0%) relative to check variety PBW725 (11.07%) (Table 1). For grain zinc concentration, range of 34.0 mg/kg to 54.0 mg/kg with mean of 43.3 mg/kg was observed among the progenies. High zinc tetraploid donor BF22 showed 50.1 mg/kg of zinc concentration while the recipient variety PBW698 carried 42.9 mg/kg grain zinc. The check variety PBW725 recorded 44.8 mg/g of zinc concentration. Nine F₃ lines were observed to be significantly superior over check variety with >50 mg/kg of zinc concentration. Significant superiority was also observed for grain iron concentration that ranged from 25.1-36.3 mg/kg with mean of 30.5 mg/kg as compared to 26.7 mg/kg for PBW698 and 32.1 mg/kg for PBW725. Progeny No. 111, 129 and 175 (Table 1) were the lines with significantly enhanced iron, zinc and protein content within the same wheat background. For grain yellow pigment content, population showed range of 2.01-4.57 ppm and 3.12 ppm of population mean value which is much lower than that of yellow pigment donor parent 'BF22' (6.62ppm). This depicts that the high yellow pigment allele from *T. durum* though introgressed in some of the BC_1F_2 backcross derivatives were lost in further crossing. Thus, this population provided a sound base for identification and derivation of the lines with enhanced protein content, iron as well as zinc concentration along with good agronomic performance. The evaluation of F₃ lines was

Table 1. Mean for agronomic and grain quality parameters recorded on promising *Gpc-B1* positive F₃ progenies from the cross PBW698/BF22//PBW698/3/ BWL3560

S.No.	Progeny no.	*Rust	Days to heading	Days to maturity	Plant height (cm)	No. of spikelets/ spike	Spike length (cm)	Tillers per m length	Grain per spike	1000-grain weight	Grain yield (g)	Grain iron conc. (mg/kg)	Grain zinc conc. (mg/kg)	Grain score	Grain protein content (%)	Yellow pigment content (ppm)
1	98	10S	90	147	105	18	11	109	37	44	160	34.2	45.1	5.7	13.92	2.91
2	111	TS	87	144	98	16	9	113	33	37	139	35.4	53.9	5.5	13.54	2.91
3	185	TS	92	149	100	20	11	80	49	29	87	35.7	47.4	5.8	13.27	3.84
4	14	TS	91	151	81	21	12	68	52	35	71	30.4	40.6	5.3	13.17	2.9
5	129	TS	89	141	109	17	10	109	35	29	134	32.9	50.5	5.4	12.93	3.23
6	12	TS	87	147	86	19	11	93	47	40	184	33.6	46.5	5.8	12.82	2.77
7	60	TS	93	145	103	18	10	100	28	39	135	30.5	48.3	5.1	12.79	2.58
8	26	TS	84	143	94	17	10	75	38	43	146	31.7	48	5.7	12.7	3.8
9	96	20S	93	146	105	19	12	82	38	41	126	32.5	42.6	5.6	12.33	2.22
10	84	10TR	90	145	102	19	11	103	47	36	174	31.8	48.2	5.3	12.31	2.92
11	132	TS	88	141	104	19	10	102	35	37	145	36.3	47.2	5.2	12.31	3.69
12	17	10S	94	151	86	21	12	104	47	43	175	29.0	50.5	5.8	12.29	3.54
13	85	TS	85	146	101	19	10	77	41	46	175	32.0	45	5.4	12.09	2.65
14	175	TS	90	147	89	20	10	104	39	34	155	32.6	54	5.5	12.09	3.54
15	65	TS	89	143	102	17	10	89	34	36	131	34.1	42.1	5	12.03	2.87
16	29	5S	78	141	88	19	10	100	37	41	161	30.6	39.7	5	12.01	3.01
17	72	TS	89	148	91	19	11	71	49	41	162	28.6	43	5.4	12.0	3.11
18	63	TS	89	145	101	19	10	69	32	45	114	32.4	46	5.5	12.0	3.45
	BF22	TS	99	151	92	21	10	74	49	38	133	25.6	50.1	5.5	10.35	6.62
	PBW698	TS	92	150	92	19	11	109	46	42	208	26.7	42.9	5.9	9.65	3.74
	BWL3560	TS	88	151	91	19	12	88	43	33	159	32.1	45	5.7	12.29	3.25
	PBW725 (check)	TS	100	155	99	22	12	104	41	44	190	32.1	44.8	6.2	11.07	2.99
	Range		78-100	141-155	81-109	16-22	9-12	68-113	28-52	29-46	71-208	25.6-36.3	39.7-54.0	5.0-6.2	9.65-13.92	2.22-6.62
	LSD(0.05)		2.27	1.55	4.46	1.93	1.22	17.56	8.47	6.84	49.79	3.42	7.45	0.27	0.97	0.39

*S = Susceptible; TS = Trace severity of susceptible type reaction; TR = Trace severity of resistant type reaction

of preliminary nature and the material is suitable for large scale evaluations.

Correlation coefficients between different traits in Gpc-B1 positive progenies

The extent to which grain protein variation is influenced by other traits in this set is brought out by the matrix of correlation coefficients given in Table 2. The highest positive influence of agronomic traits on protein content is seen to be days to flowering (r=0.225) and days to maturity (r=0.289). Days to flowering also influenced grain zinc concentration positively (r=0.265). This indicated that in late flowering lines grain protein and zinc content should be higher, though data (Table 1) showed that high protein lines were early in flowering. Data also depicted high protein F_3 progenies to be early maturing whereas correlation analysis showed their positive association. This may be because of the fact that gene *Gpc-B1* has been already reported to accelerate senescence as the physiological phenomenon in bread wheat. The check variety did not have *Gpc-B1* and hence, all progenies had early flowering compared to the check variety.

The major negative influence of protein content, zinc concentration and yellow pigment content was on grain yield (r = –0.326, –0.219 and –0.201) and 1000 grain weight (r = –0.192, –0.193 and –0.183). The negative association of grain yield with grain protein content which presents a major obstacle in their simultaneous improvement had also been earlier reported by different workers (Austin et al. 1980, Pleijel et al. 1999, Groos et al. 2003, Barneix 2007, Blanco et al. 2012, Wurschum et al. 2016, Rapp et al. 2018). In this study, most of the high protein lines were low yielding, however, some of the lines with high protein content (progeny no. 12, 17, 84 and 85) were at par to the check variety for grain yield. Thousand grain weight is a significant yield contributor and correlation matrix clearly showed negative association with protein content which indicated that *Gpc-B1* gene positive lines with relatively higher protein content had less bold grains. The enhancement in protein content did not show concomitant increase for grain zinc and iron concentration. The comparison of yield in

Table 2. Phenotypic correlation coefficients among the traits recorded on *Gpc-B1* positive F_3 progenies from the cross PBW698/BF22//PBW698/3/BWL3560

	Days to maturity	Plant height	No. of spikelets/ spike	Spike length	Tillers per m length	No. of grains/ spike	1000- grain weight	Grain yield	Grain iron conc.	Grain zinc conc.	Grain score	Grain protein content	Grain yellow pigment content
Days to heading	0.498**	0.128	0.409**	0.315**	-0.207*	0.157	-0.222*	-0.293**	0.171	0.265**	0.059	0.225*	-0.042
Days to maturity		-0.073	0.487**	0.425**	-0.334**	0.071	0.077	-0.388**	0.077	0.157	-0.028	0.289**	0.163
Plant height			-0.038	-0.026	0.205*	-0.257**	0.271**	0.181*	0.009	-0.077	0.155	0.128	-0.217*
No. of spikelets/ spike				0.613**	-0.216*	0.311**	0.048	-0.128	-0.128	-0.042	-0.034	0.036	0.007
Spike length					-0.333**	0.332**	0.013	-0.130	-0.028	-0.087	-0.072	0.099	-0.026
Tillers per m length						-0.210*	0.093	0.610**	-0.029	-0.196*	0.276**	-0.085	-0.149
No. of grains/ spike							-0.204*	-0.010	-0.200*	-0.164	0.097	-0.124	0.019
Thousand grain weight								0.215*	-0.082	-0.193*	0.005	-0.192*	-0.183*
Grain yield									-0.146	-0.219*	0.287**	-0.326**	-0.201*
Grain iron conc.										0.318**	-0.152	0.516**	0.041
Grain zinc conc.											-0.087	0.191*	0.040
Grain score												-0.291**	-0.120
Grain protein content													-0.042

*Significant at 5% level of significance, **Significant at 1% level of significance
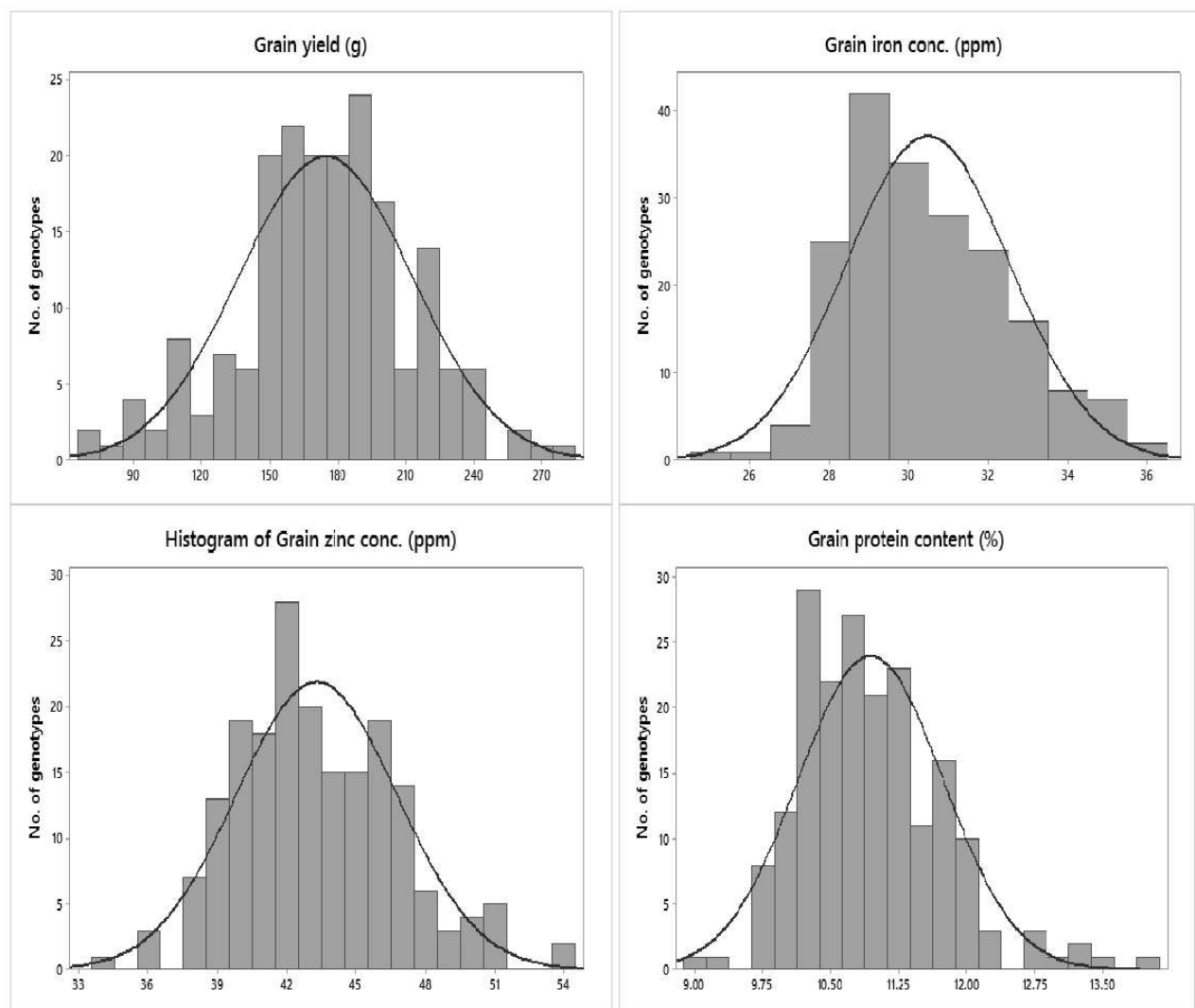
Fig. 3. Graphical distribution of 192 F$_3$ progenies from the cross PBW698/BF22/ PBW698/BWL3560 for various agronomic traits

wheat lines showed negative influence of Gpc-B1 gene and the yield reduction was routed mainly through the reduction in 1000 grain weight in Gpc-B1 positive lines (Singh et al. 2018). No significant correlation was observed for grain protein content with plant height while negative association was observed for plant height with grain yellow pigment content. Grain number per spike and tiller number showed non significant negative correlation with grain protein content. Among the quality traits, highly significant and positive correlation was estimated for grain protein with grain zinc (r=0.191) and iron (r=0.516) concentration. In the present set of F$_3$ lines, 129 lines were *Gpc-B1* gene positive but the good yielding lines were present in both gene positive and negative group. Maximum improvement for grain protein was observed in *Gpc-*

B1 positive progeny no. 98 to the tune of 44.2 % and 25.7 % over PBW725 and PBW698 respectively. In this study, *Gpc-B1* gene served as a major genetic factor for the transfer of not only the protein content but also grain micronutrient concentration. This provides an alternative approach to the more challenging minor gene based grain micronutrient improvement. Strong positive correlation of grain protein with grain zinc and grain iron concentrations provide the opportunity to improve all the three important nutritional quality traits simultaneously.

In our efforts to transfer *Gpc-B1* and high zinc concentration trait to wheat advanced line PBW698 carrying rust resistance genes (*Yr10, Yr15, Lr24* and *Lr28*), a competitive plant material has been generated

where lines with enhanced protein and micronutrient content were obtained but with significantly lowered thousand grain weight and lowered grain yield. The reduction in yield could be compensated with other yield components. In the present study, grains per ear tend to be conserved through deliberate or unconscious selection but it did not contribute significantly to yield as no correlation was observed in the set with yield. Another component i.e. number of tillers was a significant contributor to yield as indicated by significant positive correlation, but most of the progenies showed low tillering ultimately contributing towards lowered yield. So, the negative correlation of grain yield with grain protein content is the fundamental constraint, while the effects on yield contributing traits depends upon their relationship with yield in a particular set.

Therefore, *Gpc-B1* gene raised the protein and micronutrient level but it is incompatible with maintaining yield ability of an adapted, productive line and for its manifestation, flexibility for the productivity related components needs to be allowed. No correlation was observed for grain yellow pigment content with protein, iron and zinc concentration but this quality trait is also negatively associated with grain yield and thousand grain weight. Thus, it could be inferred that the improvement for four nutritional quality traits needed the yield moderation even in productive lines.

Authors' contribution

Conceptualization of research (NSB, VSS); Designing of the experiments (KK, NSB, VSS); Contribution of experimental materials (KK, NSB, PC); Execution of field/ lab experiments and data collection (KK, GSM, HK); Analysis of data and interpretation (KK, NSB); Preparation of manuscript (KK, AS, PS).

Declaration

The authors declare no conflict of interest.

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