Journal of Cereal Science 117 (2024) 103894

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Identifying QTL for grain protein content independent from grain yield-related traits in durum wheat

Domenica Nigro [a], Emanuela Blanco [b], Giacomo Mangini [b], Barbara Laddomada [c], Natalia Sgaramella [a], Massimo Antonio Signorile [a], Rosanna Simeone [a], Antonio Blanco [a,*]

[a] Department of Soil, Plant and Food Sciences (DiSSPA), University of Bari Aldo Moro, Via Amendola 165/A, 70126, Bari, Italy
[b] Institute of Biosciences and Bioresources, National Research Council (CNR), Via Amendola 165/A, 70126, Bari, Italy
[c] Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Prov.le Monteroni, 73100, Lecce, Italy

ARTICLE INFO

Original content: Identifying QTL for grain protein content independent from grain yield-related traits in durum wheat (Original data)

Keywords:
Durum wheat
Grain protein content
Grain protein deviation
QTL

ABSTRACT

Grain protein content (GPC) is a crucial quality trait determining the nutritional, rheological, and end-use properties characteristics of wheat. Breeding programs for GPC were hindered by complex genetic control, the strong influence of environmental factors, and the negative correlation between GPC and grain yield. To identify stable quantitative trait loci (QTL) associated with increased GPC without decreasing grain yield, a recombinant inbred lines population of durum wheat was genotyped with a 25K SNP array and evaluated for GPC and yield-related traits in four field trials. Six QTL for GPC were identified on chromosomes 1B, 2B (two loci), 4B, 5A, and 6A, consistently expressed across environments, four of which had antagonistic effects on grain yield per spike (GYS). Two QTL (*QGpc.mgb-5A* and *QGpc/TKW.mgb-2B.2)* on 2B and 5A chromosomes were independent of GYS variations and could be used in marker-assisted selection (MAS) for GPC improvement. Identifying and utilizing beneficial QTL/genes for wheat improvement requires careful consideration of the inverse relationship between GPC and yield-related traits, and phenotypic data collected across multiple environments. MAS or genomic selection techniques can effectively target favorable alleles for GPC enhancement while minimizing the impact on grain yield.

1. Introduction

Grain protein concentration and composition are major traits of baking properties of common wheat (*Triticum aestivum* L. ssp. *aestivum*) and of pasta-making technology characteristics of durum wheat (*Triticum turgidum* L. ssp. *durum* Desf.), as well as for the nutritional value and sensorial quality of wheat end-products. In general, high protein and gluten content or moderate protein levels with high gluten quality are associated with superior pasta cooking quality. Considering the various pasta drying methods, D'Egidio et al. (1990) found that protein content and gluten quality were both significant factors in determining the quality of cooked pasta at low drying temperatures, while only protein content was crucial at high drying temperatures. Under normal cultivation conditions, wheats have a low GPC which generally varies from 10% to 16%. During the last decades, the increase in GPC has been primarily achieved by increasing nitrogen (N) fertilization. However, the high prices of N fertilizers and the negative environmental effects of nitrate leaching and nitrous oxide emissions through denitrification have prompted interest in the possibility of reducing the total amount of N applied to cereal cropping systems while retaining the high productivity of modern cultivars.

Linkage mapping investigations using biparental populations and association mapping (Genome-Wide Association Study, GWAS) based on germplasm collections allowed the identification of several QTL controlling GPC on all wheat chromosomes (reviews by Kumar et al., 2018; Rapp et al., 2018). Genes involved in N uptake from the soil, controlling the enzymes of amino acid metabolism, or potentially involved in the transfer of N to the protein in the grain have been also considered for increasing GPC (Nigro et al., 2019). The pathway for nitrogen metabolism is complex, covering multiple steps like nitrate uptake, reduction, assimilation into amino acids, and translocation throughout the plant, each process involving numerous genes and transcription factors. The activities of the overall involved genes and enzymes could be

* Corresponding author.
E-mail addresses: domenica.nigro@uniba.it (D. Nigro), emanuela.blanco@ibbr.cnr.it (E. Blanco), giacomo.mangini@ibbr.cnr.it (G. Mangini), barbara.laddomada@ispa.cnr.it (B. Laddomada), natalia.sgaramella@gmail.com (N. Sgaramella), massimoantonio.signorile@uniba.it (M.A. Signorile), rosanna.simeone@uniba.it (R. Simeone), antonio.blanco@uniba.it (A. Blanco).

https://doi.org/10.1016/j.jcs.2024.103894
Received 7 January 2024; Received in revised form 29 February 2024; Accepted 14 March 2024
Available online 15 March 2024

Abbreviations

GPC	Grain protein content
GPD	Grain protein deviation
GY	Grain yield
GYS	Grain yield per spike
TKW	Thousand kernel weight
HD	Heading time
PH	Plant height
QTL	Quantitative trait loci
LOD	Logarithm of odds
PVE	Phenotype variance explained
GWAS	Genome-wide association study
ICIM	Inclusive Composite Interval Mapping
SNP	Single nucleotide polymorphism
MAS	Marker-assisted selection

potential targets for improving the nitrogen-use efficiency of crops. Indeed, some investigations showed the relationships between these genes and GPC QTL in bread and durum wheat (e.g. Habash et al., 2007; Nigro et al., 2019).

Multiple QTL interact with one another and with the environmental factors affecting complex traits such as GPC and grain yield. Practical breeding efforts have faced challenges in simultaneously selecting for both traits, and limited progress has been made in recent decades toward developing new wheat varieties with improved grain protein content and high grain yield. The genetical increase of GPC has been particularly hindered by the strong influence of environmental factors and by the significant negative correlation between GPC and grain yield and yield-related components in segregating populations and germplasm collections across all cereal genotypes cultivated under comparable N availability regimes (e.g., Simmonds 1995; Bogard et al., 2010). According to Munier-Jolain and Salon (2005), the physiological basis of this inverse relationship can be attributed to the competition between carbon and nitrogen for energy as well as the dilution of nitrogen by carbon-based substances. This would explain why high-yielding modern cultivars generally have lower GPC than older genotypes (Simmonds, 1995).

The majority of GPC QTL/genes were discovered in single mapping populations and/or under specific environments, and without taking into account the relationships with yield-related characteristics. To identify GPC loci without pleiotropic effects, that are not closely associated with genes for low yield-related traits, recent studies evaluated GPC and grain yield components on the same genetic materials (reviewed by Kumar et al., 2018; Nigro et al., 2019). To take into account the negative relationship between GPC and grain yield and identify genotypes with higher GPC than the expected one at the respective yield level, Monaghan et al. (2001) proposed the "grain protein deviation (GPD)" index based on the analysis of residuals from the regression of GPC on grain yield. GPD can be used to detect lines that have a positive GPD among wheat collections or segregant populations (Bogard et al., 2010) as well as genotypes with greater GPC than expected from their GY (Monaghan et al., 2001). QTL mapping for grain protein deviation in durum and bread wheat resulted in the identification of some GPC QTL independent or partially independent of grain yield (GY) which were considered for potential use in MAS to identify high GPC and high-yielding wheat genotypes (Blanco et al., 2012; Rapp et al., 2018; Nigro et al., 2019; Ruan et al., 2021). Because GPC and GY are regulated by numerous genes, QTL studies should show the interactions between QTL/genes regulating related traits, while discovering genetic sources of increased protein content without undesirable pleiotropic effects might be helpful for simultaneously enhancing GPC and GY.

The objectives of the current study were to: i) map quantitative trait loci for GPC and yield-related traits in a biparental population of durum wheat evaluated in four field trials and genotyped with a 25K SNP array; and ii) identify stable QTL/genes associated with increased protein content without decreasing grain yield. The potential to develop closely related markers to be used in marker-assisted wheat breeding can be performed by the identification of loci controlling GPC. This is expected to provide information on the genetic relationships between GPC and yield-related traits, as well as on the genetic resources available to breeders to improve technological properties and the nutritional value of wheat end-products.

2. Materials and methods

2.1. Plant materials and phenotyping

The Ethiopian durum wheat line PG2 and the Italian durum cultivar Grecale were crossed, and single F_2 plants were advanced to the F_7 generation by the single seed descent method, resulting in a segregant population of 144 recombinant inbred lines (RILs). PG2 was developed through genealogical selection from the Ethiopian landrace CI14629, kindly provided by the USDA-ARS. Grecale, an elite Italian durum cultivar, is known for its outstanding grain qualities, including a substantial presence of carotenoid pigments.

The RIL population and the parental lines were grown in four field trials at the experimental fields of the Department of Soil, Plant and Food Sciences, University of Bari, Italy, over three growing seasons (2020, 2021, and 2022) in Valenzano, Italy (designated VAL_2020, VAL_2021, and VAL_2022) and one year (designated BA_2021) in Bari, Italy. A randomized complete block design with three replications was used for each trial. In each replication, the parental lines PG2 and Grecale were replicated three times. The experimental unit was composed of a 1-m row, spaced 30 cm apart, sown with 30 germinating seeds. Standard cultivation techniques were used, and 12 g/m^2 of nitrogen (N) was applied during the growing season. Heading time (HD) was determined as the number of days from March 1–50% ear-emergence from the flag leaf ligule. Plant height (PH) was measured at maturity from the ground to the top of the spike not including the awns. Plots were hand-harvested at maturity and grain yield per spike (GYS) was determined from 10 to 12 representative spikes shelled using a micro-thresher. The thousand kernel weight (TKW) was calculated from a 15-g seed sample per plot. The grain protein concentration (GPC) was evaluated by near-infrared reflectance spectroscopy on a 3-g sample of whole-meal flour and expressed as a percentage on a dry weight basis. Indices derived from GPC were calculated for each trial on a mean basis. According to Monaghan et al. (2001), GPC indices were defined as the linear regression of GPC on GYS (GPC/GYS), TKW (GPC/TKW), PH (GPC/PH), and HD (GPC/HD).

2.2. DNA Extraction and molecular markers

The GeneElute Plant Genomic Miniprep Kit (Sigma, USA) was employed to isolate DNA from fresh leaves of both parental and recombinant inbred lines.DNA concentration and quality were checked by agarose gel-electrophoresis and NanoDrop2000 (Thermo Scientific™, Waltham, USA). DNA samples diluted to 50 ng/μL were sent to TraitGenetics GmbH (Gatersleben, Germany) for the genotyping assays with the optimized Illumina Infinium 25K wheat array developed by SGS Institut Fresenius TraitGenetics section (SGS IF TG). The 25K wheat array contains 24,145 SNPs (17,229 markers from the Illumina 20K array, 6681 markers from the 135K Axiom array, and an additional 235 candidate genes). SNP discovery was performed with the GenomeStudio Project software package (Illumina, San Diego, USA).

2.3. Genetic linkage map

A comprehensive description of the mapping procedure was

provided by Sgaramella et al. (2023). Briefly, a total of 5942 high-quality single nucleotide polymorphism (SNP) markers were retained in the data set and used for subsequent analyses after applying quality control procedures, such as filtering for marker segregation distortion ($p > 0.001$), number of missing values (20%), minor allele frequency (0.05). Seven RILs were excluded for the high percentage of missing data. The software QTL IciMapping v. 4.2 (Meng et al., 2015) was used for grouping the markers and determining the linear order of loci. Physical SNP positions on the durum wheat Svevo reference genome v1.0 (Maccaferri et al., 2019) were provided by TraitGenetics GmbH or determined by BLAST-ing the 100 bp sequences including the SNP against the durum wheat Svevo reference genome.

2.4. Statistical and QTL analyses

The software IciMapping v. 4.2 (Meng et al., 2015) was used to determine means, and descriptive statistics and to perform the analysis of variance (ANOVA) for each trait for each field experiment. The Best Linear Unbiased Estimator (BLUE) values for the combined data across trials were determined to consider the presence of random effects (environment) in the quantitative trait loci (QTL) mapping. Genetic variance, environmental variance, genotypic x environmental variance, and broad-sense heritability were all calculated using the variance component estimations. The parental line means were compared using the Student *t*-test. To discover possible correlated effects of detected GPC QTL on yield-related traits, the mean values of each trait across environments (BLUE values) of the two groups of RILs with a different genotype at the two alleles of each GPC QTL were compared by Fisher's LSD test at $p < 0.001$. The nearest SNP marker to each QTL was used for the genotype assignment to each RIL. The Pearson phenotypic correlation coefficients (*r*) for all traits for each environment and across environments were calculated.

IciMapping v. 4.2 was also used to perform QTL mapping utilizing the Inclusive Composite Interval Mapping (ICIM) methodology (Meng et al., 2015). The mean values of each phenotypic variable for each field trial and the overall mean (BLUE) across the four trials were used for the QTL analysis. QTL was declared significant when one or more markers were linked to the trait with a threshold P value of >0.001 (log10(P)

Table 1
Descriptive statistics and heritability of nine traits of the recombinant inbred lines population derived from the cross PG2 x Grecale grown at four environments.

Trait[a]	Environment	Durum wheat parental lines		RIL population					
		PG2	Grecale	Mean	Standard Error	Minimum	Maximum	Variance	h^2
GPC	VAL_2020	16.8**	13.4	15.34	1.29	12.23	18.19	1.65	0.82
	VAL_2021	16.3**	12.5	14.21	1.18	11.68	17.81	1.40	0.81
	BA_2021	18.4**	15.1	16.43	1.03	13.74	18.92	1.05	0.76
	VAL_2022	17.4**	15.6	16.31	1.22	13.87	19.53	1.49	0.83
	Across environment[b]	17.2**	14.1	15.56	0.95	13.55	18.29	0.91	0.84
GPC/GYS	VAL_2020			0.00	1.20	−2.61	2.28	1.44	0.45
	VAL_2021			0.01	1.05	−2.21	3.22	1.10	0.48
	BA_2021			0.00	1.00	−2.68	2.44	0.99	0.56
	VAL_2022			0.00	1.11	−2.07	2.58	1.23	0.20
	Across environment[b]			0.01	0.86	−1.79	2.13	0.75	0.52
GPC/TKW	VAL_2020			−0.01	1.26	−3.15	2.83	1.58	0.76
	VAL_2021			0.00	1.18	−2.50	3.69	1.39	0.80
	BA_2021			−0.01	1.00	−2.51	2.97	0.99	0.73
	VAL_2022			−0.03	1.21	−2.55	3.59	1.46	0.83
	Across environment[b]			−0.01	0.92	−1.96	3.14	0.84	0.82
GPC/PH	VAL_2020			−0.02	1.22	−3.11	2.90	1.50	0.40
	VAL_2021			−0.01	1.04	−2.18	3.36	1.08	0.44
	BA_2021			−0.01	0.97	−2.73	2.87	0.95	0.54
	VAL_2022			−0.03	1.10	−2.48	3.44	1.20	0.25
	Across environment[b]			−0.02	0.82	−1.92	2.66	0.67	0.39
GPC/HD	VAL_2020			−0.01	1.24	−3.16	2.83	1.54	0.59
	VAL_2021			0.00	1.18	−2.50	3.66	1.40	0.77
	BA_2021			−0.01	1.01	−2.82	2.48	1.03	0.68
	VAL_2022			−0.02	1.20	−2.48	2.91	1.44	0.48
	Across environment[b]			−0.01	0.95	−2.00	2.78	0.90	0.69
GYS	VAL_2020	1.86**	3.52	2.38	0.49	1.36	3.85	0.24	0.72
	VAL_2021	2.18**	4.03	2.64	0.49	1.81	4.42	0.24	0.88
	BA_2021	1.80**	2.52	1.94	0.32	1.36	2.95	0.10	0.67
	VAL_2022	1.26**	2.42	1.66	0.36	0.63	2.67	0.13	0.82
	Across environment[b]	1.78**	3.12	2.11	0.34	1.27	3.21	0.11	0.84
TKW	VAL_2020	43.7	45.5	44.32	5.72	31.05	62.20	32.76	0.87
	VAL_2021	46.8	47.3	44.90	4.87	35.57	58.85	23.75	0.86
	BA_2021	43.3	44.1	42.05	4.83	30.73	56.07	23.35	0.90
	VAL_2022	37.7*	41.5	38.06	4.49	28.36	53.57	20.13	0.89
	Across environment[b]	43.1	44.4	41.89	4.27	32.71	52.12	18.23	0.90
PH	VAL_2020	109.9**	85.6	99.01	15.60	69.00	132.50	243.25	0.91
	VAL_2021	109.2**	85.4	97.58	15.94	63.33	136.00	254.14	0.97
	BA_2021	101.6**	76.0	88.46	13.18	60.33	113.00	173.79	0.96
	VAL_2022	91.3**	77.4	86.86	12.39	62.00	110.67	153.40	0.92
	Across environment[b]	103.0**	81.1	92.45	13.26	64.42	118.41	175.84	0.94
HD	VAL_2020	22.0**	16.0	18.00	5.22	7.33	30.00	27.20	0.98
	VAL_2021	26.8**	14.2	19.57	7.59	8.33	33.00	57.62	0.99
	BA_2021	41.6**	36.7	38.70	2.90	32.00	49.00	8.44	0.97
	VAL_2022	21.4	20.9	21.34	3.83	14.00	30.00	14.68	0.97
	Across environment[b]	27.9**	21.9	27.26	4.14	19.52	37.31	17.15	0.87

** Significantly different at $p < 0.01$ with the Student's *t-test*.

[a] HD, heading date (days from April 1st); PH, plant height (cm); GYS, grain yield per spike (g); TKW, thousand kernel weight (g); GPC, grain protein content (%); GPC/HD, GPC/PH, GPC/GYS, GPC/TKW, residuals from regression of GPC on HD, PH, GYS, TKW, respectively.

[b] Across environment: overall means calculated by the Best Linear Unbiased Estimator (BLUE).

3.0). QTL are reported when significant in the mean across environments and in at least one environment to decrease the discovery of false positives. For each identified QTL, the additive effect and the percentage of phenotypic variance explained (PVE%) were estimated. The International Rules of Genetic Nomenclature for Wheat were used for QTL designation and the software MapChart v. 2.2 was used for the graphical representation of linkage groups and QTL.

3. Results

3.1. Phenotypic data and traits correlations

Grain protein content and yield-related traits (grain yield per spike, thousand-kernel weight, heading date, plant height) were evaluated in a segregating population of 144 RILs which was grown in replicated trials in four environments in southern Italy. The mean of parental lines and mean, standard error, range, genetic variance, and broad-sense heritability of the RIL population in each environment and across environments are reported in Table 1. The Student's *t*-test indicated that the parental lines were significantly different for GPC in each environment, the line PG2 always had higher values (from 16.3% to 18.4%) than those of Grecale (from 12.5% to 15.6%). The two lines were also significantly different for grain yield per spike and for plant height and heading date.

Each of the four trials showed considerable segregation for all examined traits, and the phenotypic means of the RILs were normally distributed with no appreciable skewness or kurtosis (Fig. 1). Average values of parental lines and RILs population varied between trials conducted in different environments because of the significant influence of climatic conditions. The RIL population showed mean values of GPC (from 14.2% to 16.4%) and yield-related traits that were included between the means of the parental lines in all environments (Table 1). Highly significant differences were detected in each of the four field trials among RIL genotypes for GPC, yield-related traits, and for the GPC-derived indices GPC/GYS, GPC/TKW, GPC/PH, GPC/HD determined by covariance analysis (Table S1). The correlations between the GPC mean values in different environments were significant and varied from $r = 0.46^{***}$ to $r = 0.68^{***}$ (Table S2), which was consistent with the strong environmental effects on the phenotypic expression of GPC. The combined ANOVA across environments showed significant differences for genotypes, environments, and environment × genotypes interaction for each examined trait (Table S1). The amount of variance of the different sources of variation indicated a stronger influence of the environments on GPC and yield-related traits in comparison to the genotype effects. Relatively high heritability (h^2) values (calculated by mean) were found for GPC (from 0.76 in BA_2021 to 0.83 in VAL_2022) and across environments (0.84), as well for the examined yield-related traits. The derived GPC indices GPC/TKW and GPC/HD showed h^2 values superior to 0.48, while lower h^2 values ranging from 0.20 to 0.56 and from 0.25 to 0.54 were observed for GPC/GYS and GPC/PH, respectively (Table 1).

GPC was negatively correlated with GYS and positively correlated with plant height in all four environments and across environments. GPC was also found positively correlated with TKW in two environments (VAL_2020, BARI_2021) and the mean across environments (Table 2, Table S3). As expected, GPC was always highly correlated with all GPC-derived indices in each environment and across environments.

3.2. QTL mapping

QTL were determined by the Inclusive Composite Interval Mapping (ICIM) based on the PG2 x Grecale linkage map including 5942 SNP markers described in Sgaramella et al. (2023). Putative QTL, detected in the mean across environments (BLUE) and in at least one environment, are reported in Table 3 and depicted in Fig. 2 with their main characteristics (confidence interval, adjacent markers, genetic position, additive effects, and percentage of phenotypic variance explained). Physical positions (bp) are also reported according to the durum wheat Svevo reference genome (Maccaferri et al., 2019). Five QTL for GPC (*QGPC.mgb-1B, QGPC.mgb-2B, QGPC.mgb-4B, QGPC.mgb-5A, QGPC.mgb-6A*) were detected on chromosomes 1B, 2B_2, 4B, 5A and 6A, with LOD scores ranging from 3.1 to 9.3 and PVE from 5.6% to 18.7%. The positive alleles (increased GPC) were all contributed by the Ethiopian line PG2 with allelic effects ranging from 0.22% to 0.40% (Table 3, Fig. 2).

Three QTL were detected for grain yield per spike (*QGYS.mgb-1B.1, QGYS.mgb-1B.2, QGYS.mgb-2B*) on chromosomes 1B (2 QTL) and 2B_2, four QTL for the thousand-grain weight (*QTKW.mgb-2A, QTKW.mgb-3B, QTKW.mgb-5A, QTKW.mgb-7A*) on chromosomes 2A, 3B, 5A and 7A, three QTL for plant height (*QPH.mgb-1A, QPH.mgb-4B, QPH.mgb-5A*) on chromosomes 1A, 4B and 5A, and three QTL for heading date (*QHD.mgb-1B, QHD.mgb-2A, QHD.mgb-7B*) on chromosomes 1B, 2A and 7B, (Table 3, Fig. 2).

In order to consider the genetic interdependence between GPC and yield-related traits, the QTL analysis was also performed on the residuals from the regression of GPC on yield-related traits. This analysis detected one or more QTL for GPC-derived indices located at the same genetic

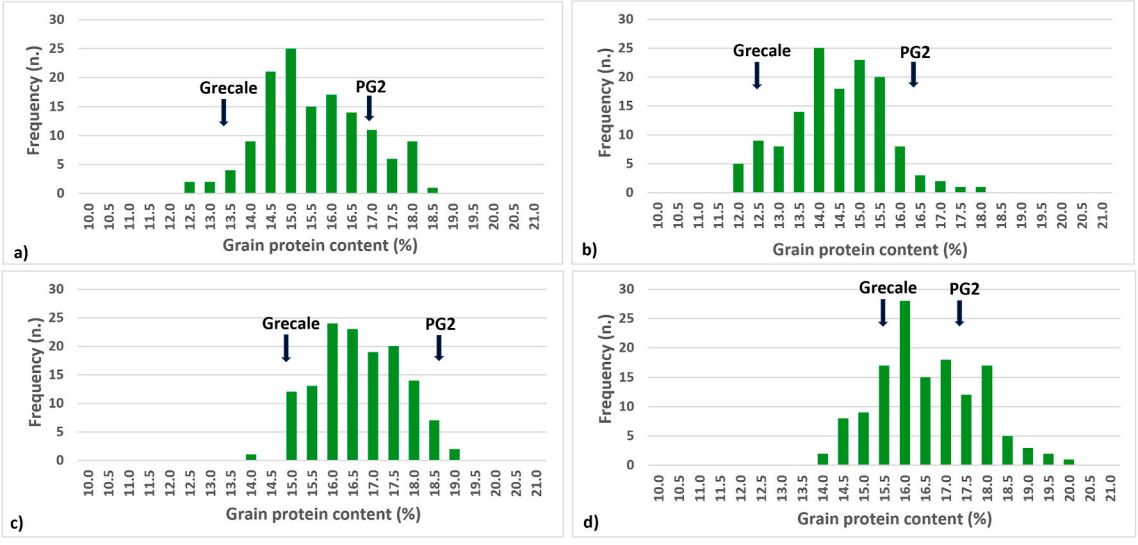

Fig. 1. Frequency distributions of the grain protein content in the recombinant inbred lines population PG2 x Grecale grown in four environments. Values represent the phenotypic mean between three field replications for each recombinant inbred line. a) VAL_2020; b; VAL_2021; c; BA_2021; a; VAL_2022.

Table 2
Phenotypic correlation coefficients using best linear unbiased estimators (BLUEs) among nine traits of the recombinant inbred lines population PG2 x Grecale grown at four environments.

Trait	HD	PH	GYS	TKW	GPC	GPC/HD	GPC/PH	GPC/GYS
PH	0.07							
GYS	0.10	−0.12						
TKW	−0.19*	0.42***	0.24**					
GPC	0.03	0.51***	−0.41***	0.28***				
GPC/HD	0.02	0.43***	−0.34***	0.21*	0.87***			
GPC/PH	0.12	0.24**	−0.29***	0.04	0.71***	0.81***		
GPC/GYS	0.09	0.36***	−0.08	0.22**	0.58***	0.64***	0.73***	
GPC/TKW	0.03	0.49***	−0.45***	0.19*	0.99***	0.86***	0.72***	0.55***

*, **, ***: significant at 0.05P, 0.01P and 0.001P, respectively.
HD, heading date (days from April 1st); PH, plant height (cm); GYS, grain yield per spike (g); TKW, thousand kernel weight (g); GPC, grain protein content (%); GPC/HD, GPC/PH, GPC/GYS, GPC/TKW, residuals from regression of GPC on HD, PH, GYS, TKW, respectively.

position or within the same confidence interval of four GPC QTL. In particular, two GPC/GYS were detected on chromosomes 4B and 5A, four QTL for GPC/TKW on chromosomes 2B_2 (two QTL), 4B and 6A, and four QTL for GPC/HD on chromosomes 2B_2, 4B, 5A and 6A (Table 3, Fig. 2). One additional QTL for GPC/TKW (*QGPC/TKW.mgb-2B.2)* was detected on the distal end of the long arm of chromosome 2B_2.

The possible correlated effects of the six detected GPC QTL on yield-related traits were also investigated by comparing the two haplotypes of each QTL for the examined trait (BLUE means across environments) by the Fisher's LSD test at $p < 0.001$. Results are reported in Fig. 3 and Table S4. As expected, highly significant differences were found between the two alleles of each GPC QTL, with mean phenotypic effects ranging from 0.35% (*QGPC.mgb-1B*) to 0.83% (*QGPC.mgb-4B*), the PG2 allele always showing the higher mean than Grecale. Differences for GYS were significant between the two haplotypes of four GPC QTL (*QGPC.mgb-1B, QGPC.mgb-2B.1, QGPC.mgb-4B, QGPC.mgb-6A),* the Grecale allele always showing the higher mean than PG2, thus indicating antagonistic effects of GPC and GYS. Interestingly no significant differences for GYS were found for the haplotypes of *QGPC/TKW.mgb-2B.2* and *QGPC.mgb-5A*. The two haplotypes of each GPC QTL showed no significant differences in TKW, PH, and HD, excluding the two haplotypes of *QGpc.mgb-4B* for TKW and PH, and *QGpc.mgb-2B.1* for PH.

3.3. Plant height and GPC

Considering the well-known effects of the semi-dwarfing genes *Rht* on grain yield, seed weight, and GPC caused by an increased partitioning of assimilates to developing spikes, the possible influence of plant height on GPC and yield-related traits was carefully analyzed in the mapping population PG2 x Grecale. The parental lines significantly differed for PH in individual environments and across environments, the PG2 line being always taller than Grecale by up to 22 cm. ICIM analysis detected three PH QTL on chromosomes 1A, 4B, and 5A, contributing 69.1% of the phenotypic variance. Most of the plant height variation could be attributed to *QPh.mgb.4B,* which was identified as the semi-dwarfing gene *Rht-B1* being mapped by the gene-derived marker *TG0010*. *Rht-B1* was significantly detected in each of the four environments and the BLUE mean across environments and accounted for 41.6–59.5% of PH variation. Based on the functional marker *TG0010*, the PH effect was estimated to be 9.4–12.3 cm in individual environments and 11.3 cm across environments. The additional detected PH loci *QPh.mgb.1A* and *QPh.mgb.5A* explained 4.5–5.1% of phenotypic variation and affected PH by 3.1 cm and −3.3 cm, respectively. Comparing the BLUE mean values of each trait of the two groups of RILs with a different genotype at *Rht-B1,* as expected we found a significant effect of the Grecale allele (*Rht-B1)* in decreasing PH, but we also found negative correlated effects on GPC, GPC/GYS and TKW and a positive effect on GYS (Fig. 3). The dominant allele *Rht-B1* was associated with a 1.00% decrease in GPC and a 0.85% decrease in GPC/GYS in the combined data. The same phenotypic effects were shown by *QPh.mgb.4B* mapped about 10 cM downstream of *Rht-B1* and its derived marker *TG0010*.

4. Discussion

4.1. QTL for grain protein content

In this study, we used a high-density SNP map to detect QTL for GPC and yield-related traits in a recombinant inbred line population of durum wheat. The RIL population showed wide variation for GPC and yield-related traits in each environment, and the combined ANOVA across environments revealed significant differences for genotypes, environments, and environment × genotype interaction for all examined traits (Table S1). GPC was always negatively correlated with GYS in all four environments and across environments. Overall, five QTL for GPC, significant across environments and in at least one environment, were detected on five chromosomes with the positive alleles (increased GPC) all contributed by the line PG2. Fourteen QTL were detected for the examined grain-yield related traits: four QTL for grain yield per spike, four for the thousand-grain weight, three for plant height, and three for heading date (Table 3, Fig. 2).

To consider the possible genetic interdependence between GPC and yield-related traits, the QTL analysis was also performed on the residuals from the regression of GPC on yield-related traits. According to Monaghan et al. (2001), such a procedure can allow a QTL analysis to be carried out independently from variation in yield-related traits. This analysis detected one or more QTL for GPC-derived indices located within the same confidence interval of the five detected GPC QTL (Fig. 2). Taking into account the GPC-derived index GPC/GYS, which considers the variations of the major yield-component trait GYS, negatively correlated with GPC, we found that the GPC QTL located on chromosomes 1B, 2B, and 6A (*QGPC.mgb-1B, QGPC.mgb-2B*, and *QGPC.mgb-6A*) failed to show significant effects. These three GPC QTL were overlapping or close to three QTL for decreasing effects on GYS (*QGYS.mgb-1B.1*, *QGYS.mgb-2B, QGYS.mgb-6A*), thus indicating comigrating loci with opposite pleiotropic effects on both traits. *QGPC.mgb-4B* was overlapping with the GPC-derived indices GPC/TKW and GPC/HD (*QGPC/TKW.mgb-4B*, *QGPC/HD.mgb-4B)* and located at 12 cM from *QGPC/GYS.mgb-4B* and *QGPC/PH.mgb-4B,* suggesting a strong interaction with the major reduced plant height *Rht-B1*. *QGPC.mgb-5A* was coincident with *QGPC/GYS.mgb-5A* and *QGPC/HD.mgb-5A,* thus indicating its independence from variation of GYS and HD. One additional QTL for GPC/TKW (*QGPC/TKW.mgb-2B.2)* was detected on the distal end of the long arm of chromosome 2B_2 most likely due to the reduction of variance in the residuals of the regression of GPC on TKW which lowers the detection threshold. A TKW QTL was detected in the same genetic interval in two environments but not reported in Table 3 based on the adopted criteria of declaring a QTL significant across environments and at least in one environment.

The possible correlated effects of the six detected GPC QTL on yield-

Table 3
Quantitative Trait Loci (QTL) for grain protein content and yield-related traits detected in the recombinant inbred line (RIL) mapping population of durum wheat PG2 x Grecale across four environments (BLUE)°.

Trait	QTL	Chrom.	Genetic position	Confidence interval	Left Marker	Right Marker	Left Marker Phys. pos.[a]	Right Marker Phys. pos.[a]	LOD	PVE	Add
			cM		ID	ID	bp	bp		(%)	
GPC	*QGPC.mgb-1B*	1B	10	9.5–10.5	AX-94961642	IWB12475	19,272,289	19,318,453	3.9	7.0	0.24
GPC	*QGPC.mgb-2B*	2B_2	38	33.5–39.5	IWB11285	IWB32005	130,360,301	134,636,889	6.3	12.7	0.32
GPC	*QGPC.mgb-4B*	4B	51	36.5–52.5	IWB23253	IWB72792	26,879,575	84,559,368	9.3	18.7	0.40
GPC	*QGPC.mgb-5A*	5A	53	51.5–53.5	AX-158564968	IWB7120	na	399,255,782	3.9	7.0	0.24
GPC	*QGPC.mgb-6A*	6A	55	54.5–56.5	AX-95025823	IWB72208	67,435,252	75,863,290	3.1	5.6	0.22
GPC/GYS	*QGPC/GYS.mgb-4B*	4B	39	37.5–52.5	IWB23253	IWB72792	26,879,575	84,559,368	10.9	12.6	0.34
GPC/GYS	*QGPC/GYS.mgb-5A*	5A	53	51.5–53.5	AX-158564968	IWB7120	na	399,255,782	7.1	7.5	0.26
GPC/TKW	*QGPC/TKW.mgb-2B.1*	2B_2	37	32.5–39.5	IWB11285	IWB32005	130,360,301	134,636,889	7.1	9.8	0.34
GPC/TKW	*QGPC/TKW.mgb-2B.2*	2B_2	201	199.5–201.5	AX-95116218	AX-94702510	763,589,992	764,393,129	4.0	4.7	0.24
GPC/TKW	*QGPC/TKW.mgb-4B*	4B	51	36.5–52.5	IWB23253	IWB72792	26,879,575	84,559,368	5.8	7.5	0.30
GPC/TKW	*QGPC/TKW.mgb-6A*	6A	55	54.5–56.5	AX-95025823	IWB72208	67,435,252	75,863,290	4.2	5.3	0.25
GPC/HD	*QGPC/HD.mgb-2B*	2B_2	38	33.5–39.5	IWB11285	IWB32005	130,360,301	134,636,889	6.6	13.2	0.33
GPC/HD	*QGPC/HD.mgb-4B*	4B	51	36.5–52.5	IWB23253	IWB72792	26,879,575	84,559,368	9.1	18.1	0.39
GPC/HD	*QGPC/HD.mgb-5A*	5A	53	51.5–53.5	AX-158564968	IWB7120	na	399,255,782	3.6	6.4	0.23
GPC/HD	*QGPC/HD.mgb-6A*	6A	55	52.5–56.5	AX-95025823	IWB72208	67,435,252	75,863,290	3.3	5.8	0.22
GYS	*QGYS.mgb-1B.1*	1B	9	5.5–9.5	IWB14060	AX-158557069	15,320,002	17,029,294	3.0	5.5	−0.08
GYS	*QGYS.mgb-1B.2*	1B	67	64.5–67.5	IWB59128	IWB35239	482,809,613	486,755,800	5.2	10.3	−0.10
GYS	*QGYS.mgb-2B*	2B_2	56	54.5–56.5	IWB4614	IWB50296	178,740,650	189,583,615	3.2	6.2	−0.08
GYS	*QGYS.mgb-6A*	6A	54	51.5–56.5	AX-158527841	AX-95025823	60,166,448	67,435,252	3.3	6.3	−0.08
TKW	*QTKW.mgb-2A*	2A	97	96.5–97.5	IWA32	IWB2234	560,730,776	568,233,451	10.4	14.5	1.63
TKW	*QTKW.mgb-3B*	3B	147	146.5–148.5	IWB10631	IWB47344	749,353,433	756,364,388	5.5	7.0	−1.13
TKW	*QTKW.mgb-5A*	5A	170	164.5–176.5	AX-110445262	IWB12226	581,455,166	595,046,558	3.8	5.6	−1.00
TKW	*QTKW.mgb-7A*	7A	32	31.5–32.5	AX-158567256	AX-158591662	40,432,035	41,335,224	4.5	5.4	−1.00
HD	*QHD.mgb-1B*	1B	168	167.5–168.5	IWB5319	AX-95199802	672,461,254	677,461,678	6.1	6.2	−1.04
HD	*QHD.mgb-2A*	2A	40	39.5–40.5	AX-158561966	IWB7033	36,543,717	40,185,401	31.5	54.5	3.08
HD	*QHD.mgb-7B*	7B	11	10.5–11.5	AX-95072534	AX-95164074	8,375,941	9,381,377	4.4	4.9	−0.91
PH	*QPH.mgb-1A*	1A	61	60.5–61.5	IWA5083	AX-158539847	363,191,947	363,486,267	4.7	4.5	3.07
PH	*QPH.mgb-4B*	4B	39	38.5–39.5	IWB23253	TG0010b	26,879,575	29,293,179	35.7	59.5	11.34
PH	*QPH.mgb-5A*	5A	168	163.5–172.5	AX-110445262	IWB12226	581,455,166	595,046,558	3.8	5.1	−3.28

Left CI and Right CI: confidence intervals for start and end respectively; LOD: Logarithm of Odds; PVE(%): Percentage of the phenotypic variance explained; Add: additive effect of a QTL, where the negative sign (−) indicates the allele from parent Grecale and the absence of sign indicates the allele from parent PG2. Suggestive QTL below the threshold (2.8 < LOD <3.0) are reported in italics.

[a] Phys. pos.: Physical position (bp) according to the durum wheat Svevo reference genome (Maccaferri et al., 2019). na: not available.

related traits were also examined through haplotype analysis using the mean of each trait across environments (BLUE values) by the Fisher's test. As expected, the comparison confirmed the significant difference between the two alleles of each GPC QTL as found by the previous ICIM analysis and indicated that four high GPC QTL alleles (*QGpc.mgb-1B, QGpc.mgb-2B.1, QGpc.mgb-4B* and *QGpc.mgb-6A*) had significant negative effects on GYS determined by loci comigrating or with opposite pleiotropic effects on both traits (the alleles for high GPC co-localize with alleles for low GYS). The two alleles of *QGpc.mgb-5A* and *QGpc/TKW.mgb-2B.2* were not significantly different for GYS, TKW, PH, and HD, and then could be considered independent from grain-yield related trait variations. Interestingly, these two GPC QTL had additive effects as

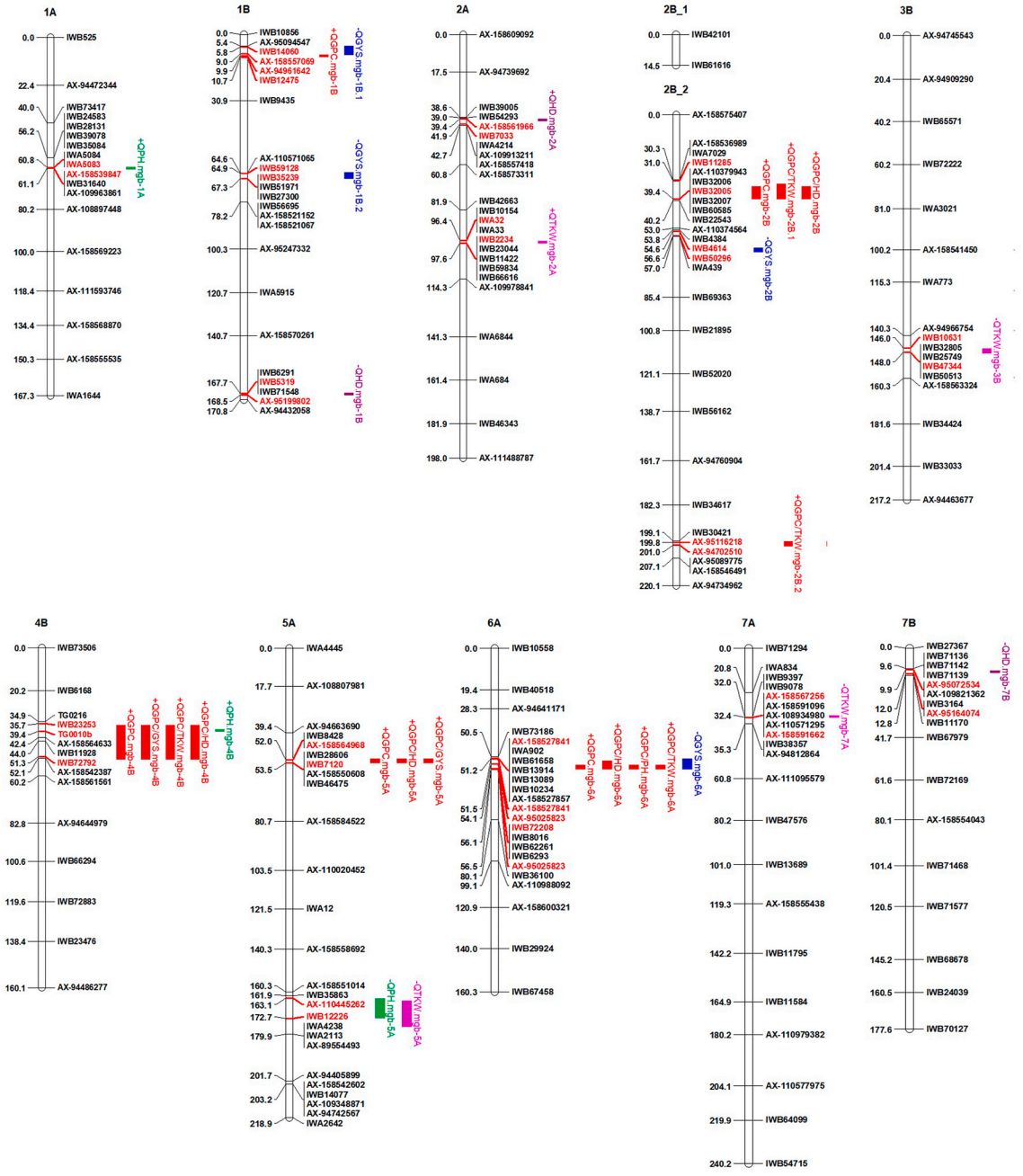

Fig. 2. Genetic map and QTL for grain protein content (GPC), grain yield per spike (GYS), thousand-kernel weight (TKW), plant height (PH), heading date (HD), and for the residuals of the regression of GPC on GYS (GPC/GYS), TKW (GPC/TKW), PH (GPC/PH), HD (GPC/HD). Each chromosome map is represented by the first and the last SNP marker and by an SNP marker every about 20 cM. Markers are indicated on the right side and cM distances are on the left side of the bar. QTL are represented by bars on the right of each chromosome. QTL names indicate the trait; the closest SNP marker to each QTL is shown in red. QTL significant across environments and at least one environment at LOD≥3.0 are reported with a positive or negative sign before the QTL name indicating the additive effect associated with an increased or decreased effect from the PG2 allele. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

shown by comparing the RILs group with both the PG2 alleles (*QGpc/TKW.mgb-2B.2_a* and *QGpc.mgb-5A_a*) with the RILs group with the Grecale alleles (*QGpc/TKW.mgb-2B.2_b* and *QGpc.mgb-5A_b)* (Table S4).

4.2. Comparison of detected QTL with previous studies and candidate genes

Identifying QTL that are shared between independent segregant populations can help to improve the confidence interval of QTL and confirm the detected effects in different genetic backgrounds. However, identifying co-located QTL among a large number of publications is difficult because different researchers have often used different types of markers in the QTL analysis and there are no consensus positions for many of the published associated markers; additionally, the mapping populations vary greatly in their level of genetic resolution. In tetraploid wheat, such difficulties can be partly overcome by using the recently developed high-quality reference genome sequences of durum wheat (Maccaferri et al., 2019) and the durum SNP-based consensus map developed by Maccaferri et al. (2014) which includes more than 30 thousand SNP markers and several hundreds of microsatellites markers

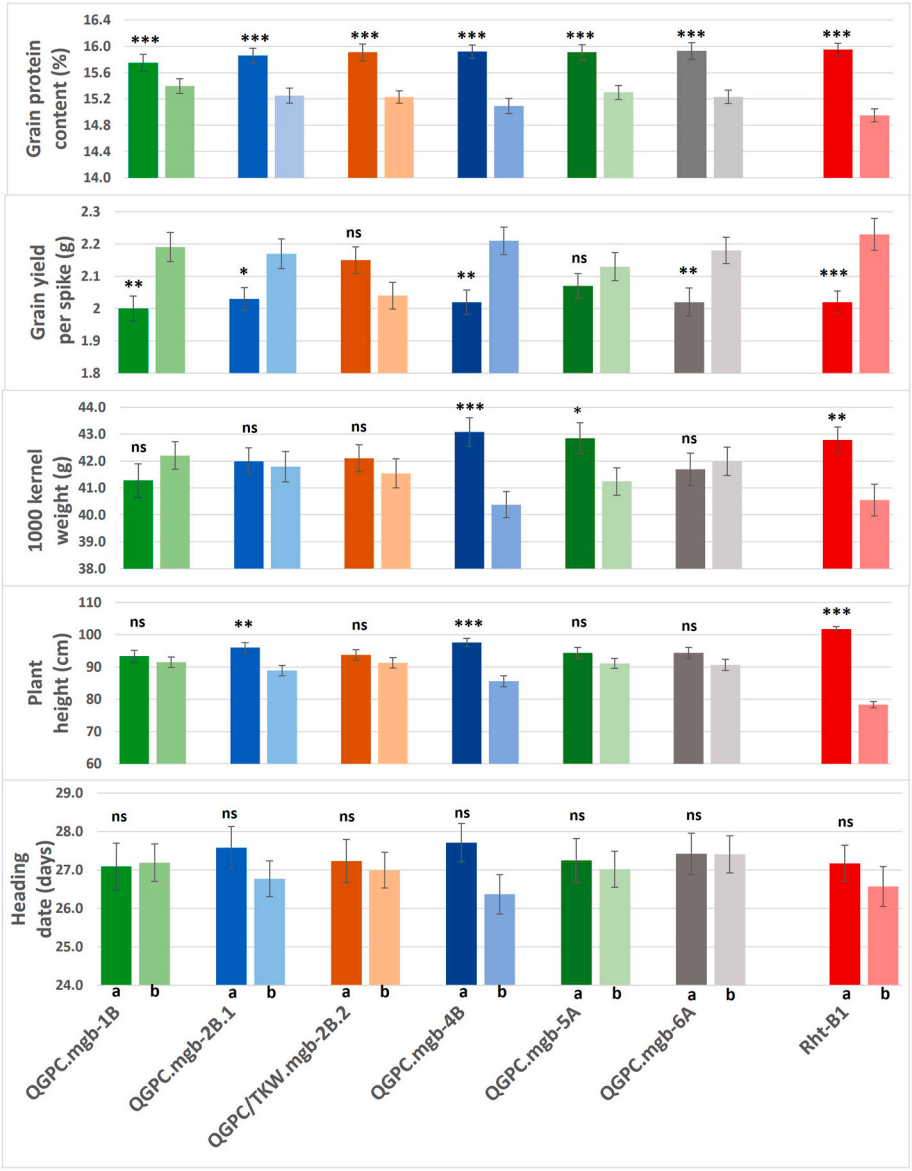

Fig. 3. Correlated effects of GPC QTL alleles and the semi-dwarfing gene *Rht-B1* on yield-related traits. a and b indicate the QTL alleles of the parents PG2 and Grecale, respectively. *, **, ***: significantly different at the 0.05, 0.01, and 0.001 probability levels, respectively, by the Fisher test. ns: not significant.

useful for comparisons with previously developed maps. By linking the genetically mapped SNP markers to the physical location of the durum wheat Svevo genome (Fig. S1), we were able to identify co-localizing QTL detected in previous studies and identify candidate genes. The GPC QTL detected in this study were putatively considered co-localized with previously reported QTL if the confidence intervals completely or partially overlapped. Dozens of genes were uncovered within each confidence interval. To narrow down the candidate genes responsible for the identified QTL, we prioritized genes and gene families that had been previously linked to GPC or GPC-derived indices in durum wheat experiments, and the high-confidence genes coding for nitrogen metabolism-related enzymes reported by Nigro et al. (2019) and Geyer et al. (2022).

4.2.1. *QGPC.mgb-1B*

QGPC.mgb-1B was physically located on chromosome arm 1BS at 19,272,289–19,318,453 bp. A GPC QTL associated with the DaRT marker wPt-0655 at 17,438,822 bp was detected by Giraldo et al. (2016) in a structured collection of *T. turgidum* including the subspecies *durum, turgidum,* and *dicoccum.* In the same region, Suprayogi et al. (2009) identified the QTL *QGpc.usw-B1* (9,706,246 bp) in a double haploid population significant in one of the six tested environments. Because of their close physical distance, the three QTL could imply the same functional gene, while they should be different from the QTL *QGpc. spa-1B.1* reported by Ruan et al. (2021) at 51,853,437 bp.

QGPC.mgb-1B was adjacent to the loci *Glu-B3* (*TRITD1Bv1G008290*, chrom. 1B: 19,410,963–19,411,436 bp) coding for low-molecular weight glutenin subunits and near to *Gli-B1* (*TRITD1Bv1G001870*, chrom. 1B: 4,313,476–4,519,819) coding for gamma-gliadin subunits. Glutenin and gliadin are the main storage proteins in wheat and exist in a wide range of variable components responsible for the gluten strength and the quality of wheat-based products. The structures, properties, and genetics of these proteins have been extensively investigated to unravel the biochemical and molecular underpinnings of their functional properties and to facilitate improvements through plant breeding, processing conditions optimization, and genetic engineering (Shewry et al., 2003).

4.2.2. *QGPC.mgb-2B.2*

QGPC.mgb-2B.2, detected on a region of about 4.3 Mb of the chromosome arm 2BS (130,360,301–134,636,889 bp), was also detected by

the GPC indices GPC/TKW and GPC/HD (*QGPC/TKW.mgb-2B.2* and *QGPC/HD.mgb-2B.2)*. The confidence interval of this QTL overlaps with the GPC and GPC/GYS QTL reported by Nigro et al. (2019) in a collection of *T. turgidum* accessions including seven subspecies, and with *QGPC.spa-2B.1* detected in the interval IWB6607– IWB22630 (126,073, 126–153,482,566 bp) in a Canadian durum population of 162 doubled haploid lines derived from the cross Pelissier × Strongfield (Ruan et al., 2021).

QGPC.mgb-2B.2 was found close to the gene *Fd-GOGAT* (*TRITD2Bv1G047890*, chrom. 2B: 120,766,653–120,782,876 bp), which encodes the enzyme ferredoxin-dependent glutamine-oxoglutarate amidotransferase involved in nitrogen metabolism. Glutamate synthase (GOGAT) plays a pivotal role in glutamate synthesis by catalyzing the transfer of the amide group of glutamines to 2-oxoglutarate, producing two molecules of glutamate. Plants harbor two forms of GOGAT based on the electron donor: ferredoxin-Fd-dependent and NADH-dependent. GOGAT, in conjunction with glutamine synthetase (GS), ensures the flow of nitrogen from $NH4^+$ to glutamine and glutamate, which are then utilized in various aminotransferase reactions for amino acid synthesis. *Fd-GOGAT* was previously found associated with GPC QTL in the Svevo x Ciccio durum RIL population evaluated in five environments and validated in near-isogenic lines (Nigro et al., 2020).

4.2.3. QGPC/TKW.mgb-2B.2

A QTL controlling the GPC/TKW index (*QGPC/TKW.mgb-2B.2*) was found on the distal end of the long arm of chromosome 2B (763,589,992–764,393,129 bp) showing an additive effect of 0.24 of GPC percentage units and 4.7% of PVE. In proximity to its confidence interval AX-95116218-AX-94702510, two QTL for GPC were previously reported in a collection of Canadian durum wheat germplasm (N'Diaye et al., 2017), and in the Canadian durum Pelissier × Strongfield mapping population (Ruan et al., 2021), respectively.

QGPC/TKW.mgb-2B.2 was at about 3 Mbp from the *GlnE* gene (*TRITD2Bv1G254550,* chrom. 2B: 760,383,403–760,390,399 bp) coding for the bifunctional glutamine synthetase adenylyltransferase/adenylyl-removing enzyme that catalyzes the initial step in ammonium assimilation, converting it into glutamine, a crucial precursor in amino acid biosynthesis.

4.2.4. QGPC.mgb-4B and Rht-B1

QGPC.mgb-4B was identified in a large confidence interval (36.5–52.5 cM, 26.9–84.6 Mbp) on the short arm of chromosome 4B also including three QTL for GPC-derived indices (*QGPC/GYS.mgb-4B, QGPC/TKW.mgb-4B,* and *QGPC/HD.mgb-4B)*. In this interval, Fatiukha et al. (2020) detected a GPC QTL in a mapping population derived from a durum × wild emmer wheat cross evaluated in five environments. The large confidence interval indicates that the positions of the two QTL are uncertain, and therefore it cannot be definitively determined whether they represent the same functional gene or different QTL.

Among the several HC genes annotated within this 4BS region, the semi-dwarfing reduced height (*Rht-B1*) and the teosinte branched1 (*TB1-B1*) genes were found. The *Rht-1* genes established the beginning of the green revolution in wheat by decreasing plant height and increasing productive tillers. *Rht-1* encodes for a DELLA (aspartic acid–glutamic acid–leucine–leucine–alanine) protein. Two widely used allelic variants (*Rht-B1b* and *Rht-D1b)* confer no responsiveness to gibberellic acid, both reducing plant height by 20% and increasing grain yield by 5–10%. In addition to several other pleiotropic effects, *Rht-1* variants were shown to negatively affect grain weight and GPC (Fowler et al., 2016). Considering that the cultivated parental line Grecale possesses the *Rht-B1* dwarf allele, while the tall line PG2 has the recessive allele, it is reasonable to infer that this gene played a substantial role in shaping several bio-agronomic traits in the examined biparental population. In this study, a substantial portion of PH variation was attributed to the identified QTL *QPH.mgb-4B*, which was tightly linked to the gene-derived marker *TG0010*, ultimately confirming its association with the semi-dwarfing gene *Rht-B1*. The effects of *Rht-B1* were consistently observed across all four field trials, with an estimated effect of 11.3 cm across environments and 59.5% of PVE. Although *QGPC.mgb-4B* was mapped downstream of the semi-dwarfing gene *Rht-B1* and its functional marker *TG0*010 by 22.1 Mbp, *Rht-B1* remains the most likely candidate gene.

Another important gene, the teosinte branched 1 TB-*B1 (TRITD4Bv1G012050,* chrom. 4B: 28,796,177–28,797,241*)*, closely linked to *Rht-B1*, was demonstrated to play a key role in regulating plant architecture and inflorescence development, including height and tillering (Dixon et al., 2020). Wheat genotypes expressing higher levels of *TB-1* were found to show an extra-branched phenotype due to a slowed development of meristem tissue, with paired spikelets at each floret rather than one (Dixon et al., 2020).

Interestingly, another putative candidate gene, *GSe-4B,* encoding for the cytosolic glutamine synthetase enzyme (*TRITD4Bv1G013920*, chrom. 4B: 33,110,340–33,112,982 bp) is localized within the confidence interval of *QGPC.mgb-4B* and at about 3.9 Mb downstream to *Rht-B1*. *GSe* facilitates the ATP-driven conversion of ammonium into glutamate (Glu) to generate glutamine (Gln). Because of its crucial function in absorbing and utilizing ammonium ($NH4^+$), glutamine synthetase has become a significant focus of crop research aimed at enhancing nitrogen use efficiency (NUE) (Li et al., 2011). *GSe-4B* was previously identified to be associated with GPC (Gadaleta et al., 2013) and GPD (Nigro et al., 2019) in durum wheat and with GPC in bread wheat (Geyer et al., 2022).

4.2.5. QGPC.mgb-5A

QGPC.mgb-5A, detected in the confidence interval AX-158564968-IWB7120 on the chromosome arm 5AL, was found coincident with *QGPC/GYS.mgb-5A* indicating that the expression of GPC is independent from GYS variation. As the left flanking marker AX-158564968 was not mapped on the Svevo reference genome, the closest and comigrating one at 52.0 cM (IWB8428) in the PG2 x Grecale map was considered to delimit the physical confidence interval (395,919,086–399,255,782 bp). Within this interval, a GPC QTL (*QGpc.mgb-5A.1*) associated with IWB28350 was previously detected in the durum Duilio x Avonlea RIL mapping population (Marcotuli et al., 2017).

The confidence interval of *QGPC.mgb-5A* covered a region of 3.3 Mb in which 41 high-confidence genes were detected. Among them, 8 genes encoding for glutamate receptors and one for S-adenosyl-L-methionine-dependent methyltransferases superfamily protein were noteworthy. These candidate genes are potentially involved in the carbon and nitrogen (C/N) balance during plant growth. While carbohydrates provide the energy and the carbon skeletons necessary for ammonium assimilation during amino acid biosynthesis, amino acids and proteins represent the cells' key building blocks. So far, the two metabolisms are coordinated with each other during the plant's entire life cycle through a sophisticated regulatory system controlling the expressions of genes involved in both C and N pathways. Indeed, plant C/N status can influence the expression of several genes, including the ones responsible for the biosynthesis of key-role molecules, such as sucrose (Suc), glucose (Glc), 2-oxoglutarate, glutamine (Gln), glutamate (Glu), NO_3^-, and NH_4^+ (Goel et al., 2016).

4.2.6. QGPC.mgb-6A

Chromosome arm 6AS contained a region with overlapping QTL for GPC and the derived indices GPC/TKW, GPC/PH and GPC/HD, but it also comprised a QTL for decreasing GYS. *QGPC.mgb-6A* was detected in a physical interval (chrom. 6A: 67,435,252–75,863,290 bp) including the gene *Gpc-A1* (*TRITD6Av1G032560*, 75,452,544–75,454,973 bp), a putative NAC transcription factor (*NAM-A1*). Four genes (*Gpc-A1, Gpc-B1, Gpc-A2,* and *Gpc-B2*) were found to be located on the homoeologous chromosome groups 2 and 6 in tetraploid wheat and shown to play a role in nutrient remobilization, the process of moving nutrients from senescing leaves to developing grains during grain filling (Avni et al.,

2014). *Gpc-B1*, a homolog of *Gpc-A1*, was identified and cloned by Uauy et al. (2006) and found to encode a NAC transcription factor (*NAM-B1*) that regulates GPC in wheat. The wild-type allele of this gene is associated with elevated GPC, accelerated plant senescence, and higher Zn and Fe levels in grains compared to the mutant allele However, its impact on grain weight and grain yield varies across different environments and genetic backgrounds (Avni et al., 2014). *Gpc-A1* may have the same pleiotropic effects on yield as *Gpc-B1*, as evidenced by the fact that a GYS QTL (*QGYS.mgb-6A)* with antagonistic effects with GPC was detected in the same region.

5. Conclusions

Grain protein content is a crucial trait that significantly impacts the pasta-making characteristics and baking performance of durum wheat, directly influencing the nutritional benefits of durum-based products. In this study, GPC and yield-related traits were evaluated in a RIL population in replicated trials across four environments. The combined ANOVA showed significant differences for genotypes, environments, and environment × genotype interaction for each trait; however, the amount of variance of the different sources of variation indicated a stronger influence of the environments on GPC and yield-related traits in comparison to the genotype effects. GPC was negatively correlated with GYS and positively correlated with TKW and PH in all four environments and across environments. Six GPC QTL were identified that were consistently expressed in the mean across all environments and at least in one environment. These QTL consistently exhibited relatively low additive effects and explained small or medium amounts of phenotypic variance. Remarkably, some QTL co-localized with N-related candidate genes. Two out of six detected GPC QTL were significantly associated with increased grain protein deviation, suggesting that selecting for GPC may not necessarily affect final grain yield per spike. These findings highlight that identifying and utilizing beneficial QTL/genes for GPC improvement in commercial durum wheat cultivars requires careful consideration of the generally inverse relationship between GPC and yield-related traits. Additionally, marker-trait association analysis should be conducted on phenotypic data collected across multiple environments to identify stable QTL that could be effectively used in breeding programs. Marker-assisted selection or genomic selection techniques can effectively target favorable alleles for GPC enhancement while minimizing the impact on grain yield.

Author's contribution statement

AB, DN and RS conceived the idea. NS, DN, MAS, BL, GM and EB performed the experiments and recorded observations. AB & GM analyzed the data. AB & DN wrote the primary draft, which was further augmented, edited, and improved by AB, RS, BL, EB & DN. All the authors read and approved this article for publication.

Funding

This research was partially funded by the project SaveGrain CER (Biodiversità dei cereali antichi pugliesi per la sostenibilità e della qualità), PSR Puglia 2014–2022, Italy (Mis. 10.2, operazione 10.2.1, Dds: 24250009594 - CUP B97H22003700009), and by the European Union Next-Generation EU (Piano Nazionale di Ripresa e Resilienza (PNRR)—Missione 4 Componente 2, Investimento 1.4—D.D. 1032 June 17, 2022, CN00000022). This manuscript reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

CRediT authorship contribution statement

Domenica Nigro: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Emanuela Blanco:** Writing – review & editing, Formal analysis, Data curation. **Giacomo Mangini:** Formal analysis, Data curation. **Barbara Laddomada:** Writing – review & editing, Formal analysis. **Natalia Sgaramella:** Data curation. **Massimo Antonio Signorile:** Data curation. **Rosanna Simeone:** Writing – review & editing, Conceptualization. **Antonio Blanco:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Identifying QTL for grain protein content independent from grain yield-related traits in durum wheat (Original data) (IRIS)

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2024.103894.

References

Avni, R., Zhao, R., Pearce, S., Jun, Y., Uauy, C., Tabbita, F., et al., 2014. Functional characterization of GPC-1 genes in hexaploid wheat. Planta 239, 313–324.

Blanco, A., Mangini, G., Giancaspro, A., Giove, S., Colasuonno, P., Simeone, R., et al., 2012. Relationships between grain protein content and grain yield components through quantitative trait locus analyses in a recombinant inbred line population derived from two elite durum wheat cultivars. Mol. Breed. 30, 79–92.

Bogard, M., Allard, V., Brancourt-Hulmel, M., Heumez, E., Machet, J.M., Jeuffroy, M.H., et al., 2010. Deviation from the grain protein concentration–grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. J. Exp. Bot. 61 (15), 4303–4312.

D'egidio, M.G., Mariani, B.M., Nardi, S., Novaro, P., Cubadda, R., 1990. Chemical and technological variables and their relationships: a predictive equation for pasta cooking quality. Cereal Chem. 67 (3), 275–281.

Dixon, L.E., Pasquariello, M., Boden, S.A., 2020. Teosinte Branched1 regulates height and stem internode length in bread wheat. J. Exp. Bot. 71 (16), 4742–4750.

Fatiukha, A., Filler, N., Lupo, I., Lidzbarsky, G., Klymiuk, V., Korol, A.B., et al., 2020. Grain protein content and thousand kernel weight QTLs identified in a durum× wild emmer wheat mapping population tested in five environments. Theor. Appl. Genet. 133, 119–131.

Fowler, D.B., N'diaye, A., Laudencia-Chingcuanco, D., Pozniak, C.J., 2016. Quantitative trait loci associated with phenological development, low-temperature tolerance, grain quality, and agronomic characters in wheat (*Triticum aestivum* L.). PLoS One 11 (3), e0152185.

Gadaleta, A., Nigro, D., Marcotuli, I., Giancaspro, A., Giove, S.L., Blanco, A., 2013. Isolation and characterization of cytosolic glutamine synthetase (*GSe*) genes and association with grain protein content in durum wheat. Crop Past. Sci. 65 (1), 38–45.

Geyer, M., Mohler, V., Hartl, L., 2022. Genetics of the inverse relationship between grain yield and grain protein content in common wheat. Plants 11 (16), 2146.

Giraldo, P., Royo, C., González, M., Carrillo, J.M., Ruiz, M., 2016. Genetic diversity and association mapping for agro-morphological and grain quality traits of a structured collection of durum wheat landraces including subsp. *durum, turgidum and diccocon*. PLoS One 11 (11), e0166577.

Goel, P., Bhuria, M., Kaushal, M., Singh, A.K., 2016. Carbon: nitrogen interaction regulates expression of genes involved in N-uptake and assimilation in *Brassica juncea* L. PLoS One 11 (9), e0163061.

Habash, D.Z., Bernard, S., Schondelmaier, J., Weyen, J., Quarrie, S.A., 2007. The genetics of nitrogen use in hexaploid wheat: N utilization, development, and yield. Theor. Appl. Genet. 114, 403–419.

Kumar, A., Jain, S., Elias, E.M., Ibrahim, M., Sharma, L.K., 2018. An overview of QTL identification and marker-assisted selection for grain protein content in wheat. In: Sengar, R., Singh, A. (Eds.), Eco-friendly Agro-Biological Techniques for Enhancing Crop Productivity. Springer, Singapore.

Li, X.P., Zhao, X.Q., He, X., Zhao, G.Y., Li, B., Liu, D.C., et al., 2011. Haplotype analysis of the genes encoding glutamine synthetase plastic isoforms and their association with nitrogen-use-and yield-related traits in bread wheat. New Phytol. 189 (2), 449–458.

Maccaferri, M., Cane, M.A., Sanguineti, M.C., Salvi, S., Colalongo, M.C., Massi, A., et al., 2014. A consensus framework map of durum wheat (*Triticum durum* Desf.) suitable for linkage disequilibrium analysis and genome-wide association mapping. BMC Genom. 15, 1–21.

Maccaferri, M., Harris, N.S., Twardziok, S.O., Pasam, R.K., Gundlach, H., Spannagl, M., et al., 2019. Durum wheat genome highlights past domestication signatures and future improvement targets. Nat. Genet. 51 (5), 885–895.

Marcotuli, I., Gadaleta, A., Mangini, G., Signorile, A.M., Zacheo, S.A., Blanco, A., et al., 2017. Development of a high-density SNP-based linkage map and detection of QTL for β-glucans, protein content, grain yield per spike and heading time in durum wheat. Int. J. Mol. Sci. 18 (6), 1329.

Meng, L., Li, H., Zhang, L., Wang, J., 2015. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J 3, 269–283.

Monaghan, J.M., Snape, J.W., Chojecki, A.J.S., Kettlewell, P.S., 2001. The use of grain protein deviation for identifying wheat cultivars with high grain protein concentration and yield. Euphytica 122, 309–317.

Munier-Jolain, N.G., Salon, C., 2005. Are the carbon costs of seed production related to the quantitative and qualitative performance? An appraisal for legumes and other crops. Plant Cell Environ. 28 (11), 1388–1395.

N'Diaye, A., Haile, J.K., Cory, A.T., Clarke, F.R., Clarke, J.M., Knox, R.E., Pozniak, C.J., 2017. Single marker and haplotype-based association analysis of semolina and pasta color in elite durum wheat breeding lines using a high-density consensus map. PLoS One 12 (1), e0170941.

Nigro, D., Gadaleta, A., Mangini, G., Colasuonno, P., Marcotuli, I., Giancaspro, A., et al., 2019. Candidate genes and genome-wide association study of grain protein content and protein deviation in durum wheat. Planta 249, 1157–1175.

Nigro, D., Fortunato, S., Giove, S.L., Mazzucotelli, E., Gadaleta, A., 2020. Functional validation of glutamine synthetase and glutamate synthase genes in durum wheat near-isogenic lines with QTL for high GPC. Int. J. Mol. Sci. 21 (23), 9253.

Rapp, M., Lein, V., Lacoudre, F., Lafferty, J., Müller, E., Vida, G., et al., 2018. Simultaneous improvement of grain yield and protein content in durum wheat by different phenotypic indices and genomic selection. Theor. Appl. Genet. 131, 1315–1329.

Ruan, Y., Yu, B., Knox, R.E., Zhang, W., Singh, A.K., Cuthbert, R., et al., 2021. Conditional mapping identified quantitative trait loci for grain protein concentration expressing independently of grain yield in Canadian durum wheat. Front. Plant Sci. 12, 642955.

Sgaramella, N., Nigro, D., Pasqualone, A., Signorile, M.A., Laddomada, B., Blanco, A., 2023. Genetic mapping of flavonoid grain pigments in durum wheat. Plants 12 (8), 1674.

Shewry, P.R., Halford, N.G., Lafiandra, D., 2003. Genetics of wheat gluten proteins. Adv. Genet. 49, 111–184.

Simmonds, N.W., 1995. The relation between yield and protein in cereal grain. J. Sci. Food Agric. 67 (3), 309–315.

Suprayogi, Y., Pozniak, C.J., Clarke, F.R., Clarke, J.M., Knox, R.E., Singh, A.K., 2009. Identification and validation of quantitative trait loci for grain protein concentration in adapted Canadian durum wheat populations. Theor. App. Genet. 119, 437–448.

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., Dubcovsky, J., 2006. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314, 1298–1301.