

UNIVERSIDAD POLITÉCNICA DE MADRID
Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de
Biosistemas



**Developing and analyzing experimental
populations to uncover the genetic
control of wheat quality**

DOCTORAL THESIS

Submitted for the degree of Doctor by:

Naima Bouabdellah

Master en Ciencias Alimentarias

Madrid, 2025



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**Doctoral Degree in Biotechnology and Genetic Resources of
Plants and Associated Microorganisms**

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To my parents, whose dreams and pride I carried with me on this journey — this achievement is yours as much as it is mine.

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Abstract

Until now, the genetic improvement of economically important species like wheat (*Triticum spp.*) has primarily focused on increasing yield. However, in recent years, there has been growing interest in improving end-use quality, which allows for a higher market price. Wheat quality is a broad concept, and although the important role of prolamins (glutenins and gliadins) in wheat quality has been known for a long time, the alleles of these proteins do not allow for unequivocal predictions.

The general objective of this thesis is to develop strategies that facilitate the prediction of wheat end-use quality and the identification genomic regions involved the genetic control of those traits, in order to provide advanced tools for breeding programs. To achieve this, i) a new protocol for the quantification of glutenins has been developed, ii) an intra-varietal biotype analysis in bread wheat was performed, and iii) a genomic, genetic, and phenotypic analysis of a segregating population in durum wheat was conducted.

The developed protocol is accessible to researchers with some experience in glutenin allele characterization and allows for simultaneous genotyping and quantification. The new methodology shows a high correlation with standard quantification protocols based on HPLC, but at a significantly lower cost. This protocol has been successfully employed for the development of the rest of the thesis.

The analysis of intra-varietal biotypes has confirmed that the *GLU-D1* locus is the primary determinant of quality, the amount of high-molecular-weight glutenins, and the ratio between different fractions of these proteins. To improve wheat quality in genotypes with the same low-molecular-weight glutenin composition, breeding programs should prioritize: increasing grain protein levels, high- and low-molecular-weight glutenins in genotypes with *Glu-D1a*; improving grain protein levels, high-molecular-weight glutenins, and the ratio of high-molecular-weight glutenins to low-molecular-weight glutenins in genotypes with *Glu-D1c* and increasing high-molecular-weight glutenin content and the ratio of high-molecular-weight glutenins to low-molecular-weight glutenins in genotypes with *Glu-D1l*.

The characterization of the segregating population developed in durum wheat revealed transgressive phenotypes, showing the utility of this resource for

identifying genomic regions associated with traits of interest. High-throughput genotyping enabled the development of a high-density genetic map that includes over 2,000 molecular markers and allows for fine genetic analysis.

The QTL mapping in the durum wheat population has identified a total of 10 stable QTLs associated with the control of quality, thousand-grain weight, and grain appearance. The QTLs located on chromosomes 1B, 4B, and 7A co-localize with previously described genes. The QTLs located on chromosomes 3B, 4A, and 6B open the door to the identification of new genes responsible for controlling quality and yield in durum wheat.

The analysis of genes that control carotenoid synthesis and degradation in wheat has determined that durum wheat breeding programs for pasta color should consider both processes. A good selection strategy in breeding programs should combine alleles that increase carotenoid synthesis with alleles that reduce their oxidation, as in the case of the genotype *LPX-B1.1c: Psy1-A11*.

The analyses performed in this thesis provide wheat breeders with new protocols and selection strategies for improving quality, while also opening the door for researchers to tackle positional cloning of new key genes.

Resumen

Hasta la actualidad la mejora genética de especies de alta importancia económica como el trigo (*Triticum spp.*) se ha centrado fundamentalmente en aumentar el rendimiento. Sin embargo, en últimos tiempos se ha ido incrementado el interés por la mejora de la calidad, lo que permite encarecer dicho producto en el mercado. La calidad en trigo es un concepto muy amplio, y aunque se conoce desde hace tiempo el importante papel de las prolaminas (gluteninas y gliadinas) en la calidad en trigo, los alelos de dichas proteínas no permiten predecir de manera inequívoca la calidad.

El objetivo general de la presente tesis es desarrollar metodologías que permitan predecir la calidad del trigo e identificar regiones genómicas implicadas en el control de la calidad, con el fin de proporcionar herramientas avanzadas para programas de mejora. Para ello, i) hemos desarrollado un nuevo protocolo para la cuantificación de gluteninas, ii) realizado un análisis de biotipos intra-varietales en trigo blando, iii) analizado a nivel genómico, génico y fenotípico una población segregante en trigo duro.

El protocolo desarrollado es accesible para investigadores con experiencia en caracterización de alelos de gluteninas y permite realizar de manera simultánea el genotipado y la cuantificación. La nueva metodología muestra una alta correlación con los protocolos de cuantificación estándar basados en HPLC, pero con un coste significativamente menor. Dicho protocolo ha sido empleado con éxito para el desarrollo del resto de la tesis.

El análisis de los biotipos intra-varietales ha permitido confirmar que el locus *GLU-D1* es el principal determinante de la calidad, la cantidad de gluteninas de alto peso molecular y la relación entre diversas fracciones de dichas proteínas. Para mejorar la calidad panadera en genotipos con la misma composición de gluteninas de bajo peso molecular en programas de mejora se deberá priorizar: aumentar los niveles de proteína en el grano, gluteninas de alto y bajo peso molecular en los genotipos con *Glu-D1a*; mejorar los niveles de proteína en el grano, gluteninas de alto peso molecular y la relación de gluteninas de alto peso molecular frente a bajo peso molecular en los genotipos con *Glu-D1c*; y aumentar el contenido gluteninas de alto peso molecular y la relación de gluteninas de alto peso molecular frente a bajo peso molecular en los genotipos con *Glu-D1l*.

La caracterización de la población segregante desarrollada en trigo duro ha revelado fenotipos transgresivos y mostrando la utilidad de este recurso para la identificación de regiones genómicas asociadas a caracteres de interés. El genotipado de alto rendimiento ha permitido desarrollar un mapa genético de alta densidad que incluye más de 2000 marcadores moleculares y posibilita la realización de análisis genéticos finos.

El cartografiado de QTL en la población de trigo duro ha permitido identificar un total de 10 QTLs estables asociadas con el control de la calidad, peso de mil granos y aspecto del grano. Los QTLs localizados en los cromosomas 1B, 4B y 7A co-localizan con genes previamente descritos. Los QTLs localizados en los cromosomas 3B, 4A y 6B, abren la puerta a la identificación de nuevos genes responsable del control de la calidad y rendimiento en trigo duro.

El análisis de los genes que controlan la síntesis y degradación de carotenoides en trigo ha permitido determinar que los programas de mejora de trigo duro para el color amarillo de la pasta deben tener en cuenta ambos procesos. Una buena estrategia de selección en programas de mejora deberá combinar alelos que aumenten la síntesis de carotenoides con alelos que reduzcan la oxidación de los mismos, como el caso del genotipo *LPX-B1.1c:Psy1-A11*.

Los análisis realizados en la presente tesis proporcionan a los mejoradores de trigo nuevos protocolos y estrategias de selección para la mejora de la calidad, a la vez que abren la puerta a los investigadores para abordar el clonaje posicional de nuevos genes de interés.

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Abbreviations and Acronyms

DHs	Doubled Haploid Lines
GMP	Glutenin Macropolymers
GS	Glutenin Subunits
HMW-GS	High Molecular Weight Glutenins
LMW-GS	Low Molecular Weight Glutenins
MAS	Marker Assisted Selection
M	Monomeric
MMW	Medium Molecular Weight
MW	Molecular Weight
NILs	Near Isogenic Lines
NIR	Near-Infrared Spectroscopy
P	Polymeric
QTL	Quantitative Trait Loci
RILs	Recombinant inbred lines
RP-HPLC	Reversed Phase High Performance Liquid Chromatography
SDS	Sodium Dodecyl Sulphate
SDSPAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
SDSS	Sodium Dodecyl Sulphate Sedimentation Volume Test
SE-HPLC	Size Exclusion High Performance Liquid Chromatography
SSD	Single Seed Descent
UPP	Polymeric Protein Unextractable In 0.5% SDS
UV	Ultraviolet

1. Introduction

1.1. Bread wheat and durum wheat, importance, uses, and differences

Wheat is an important cereal food crop that belongs to the grass family (*Poaceae*) (Petersen et al., 2006) and serves as a vital source of calories, providing around 20% of the world's calorie intake (Shewry & Hey, 2015). Its unrivalled adaptability and extensive cultivation make it exceptionally versatile, especially under different growing conditions in temperate climates (Marcotuli et al., 2022). The global production of wheat in 2023 amounted to 777 million tons grown on 220 million hectares, with the main production centres in China, India, Russia, and the United States of America (FAOSTAT, 2024) (Figure 1).

The main species are hexaploidy bread wheat (*Triticum aestivum* L.; $2n = 6x = 42$ chromosomes; AABBDD), which represents around 95% of global wheat production, and tetraploid durum wheat (*Triticum turgidum* L. *ssp. durum* (Desf.) Husn.; $2n = 4x = 28$ chromosomes; AABB). Durum wheat is grown on more than 17 million hectares worldwide, yielding a total production of around 40 million tons (Martínez-Moreno et al., 2022). The European Union stands out as the leading producer, with a production of 8 million tons in 2021. Noteworthy, contributors to this production include Italy, Turkey, France, and Spain, each producing a close to 1 million tons on 0.26 million hectares of land (EUROSTAT, 2023).

Wheats are the result of a natural fusion of different species (Figure 2). Initially, a major polyploidization event occurred in which the genetic material of two diploid species was fused. One species related to the wild species *Triticum urartu* (AA genome; $2n=2x=14$) (Petersen et al., 2006, Marcussen et al 2014) and the other species probably related to *Aegilops speltoides* (BB genome; $2n=14$). This fusion led to the formation of the allotetraploid *T. turgidum subsp. dicoccoides* (AABB genome; $2n=4x=28$), the ancestor of wild emmer and *T. turgidum sp. durum*, which is an integral part of the human diet. Later, a second hybridization event occurred between *T. turgidum* and *Aegilops tauschii* (DD genome), resulting in the hexaploid progenitor *T. aestivum* (AABBDD genome; $2n=6x=42$) (Nesbitt and Samuel, 1996; Petersen et al., 2006; Marcussen et al. 2014), which is now widely cultivated worldwide.

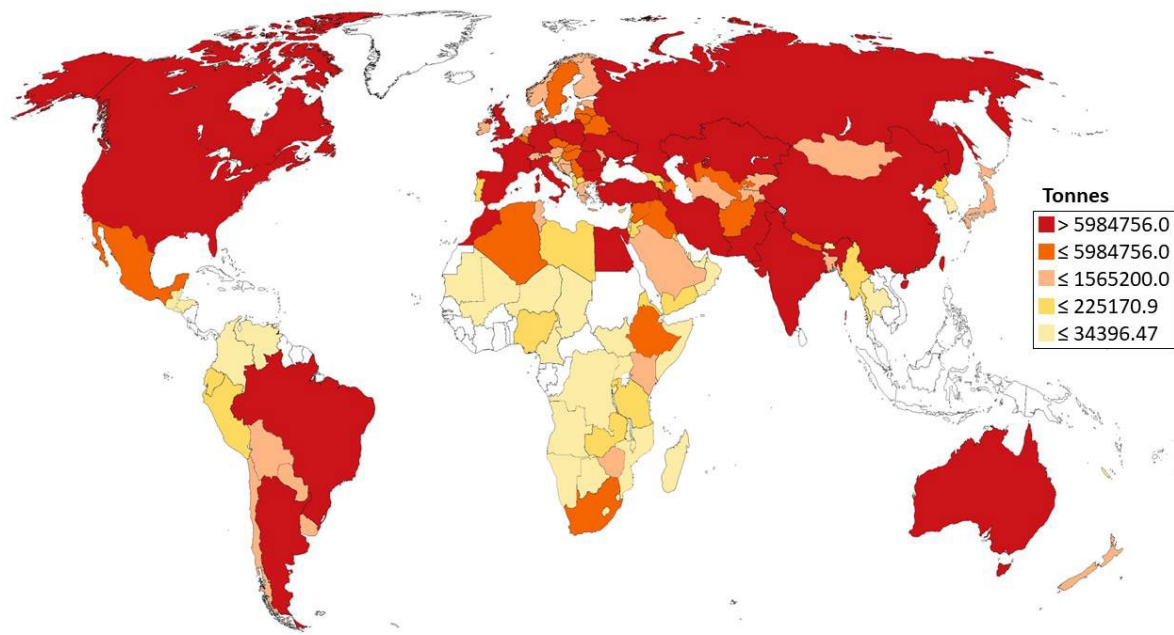


Figure 1: Wheat production worldwide in 2021

Source: adapted from FAOSTAT

In addition to the difference in terms of genome structure, bread and durum wheat also differ in terms of grain composition and quality characteristics for end use, since durum wheat contains a harder endosperm than hard-grained bread wheat (Peña et al., 2000). Bread wheat is usually ground into flour and is used to make various flat and sourdough breads, biscuits, and other baked goods (Peña et al., 2008). In contrast, durum wheat is generally ground to semolina, which is then used to make pasta, couscous, and bulgur for food production (Shewry, 2009). Wheat-derived food play a crucial role in ensuring food security, as it is a low-cost source of nutrition for a large part of the world's population, especially for millions of middle to low-income people. (Peña-Bautista et al., 2017).

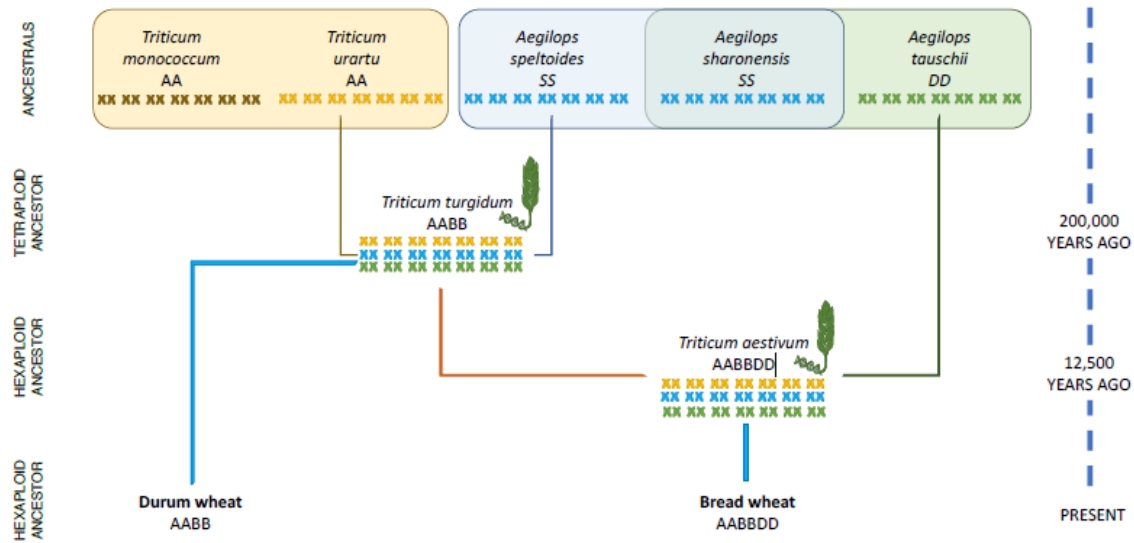


Figure 2: The origin of wheat

Source: IWGSC (2014).

1.2. Wheat quality traits and methods to estimate wheat quality

In the commercial wheat system, to be considered valuable, wheat must generally meet three basic requirements: it must have inherent qualities suitable for its intended use, be relatively free of foreign matter and impurities, and be presented in such a way that purchasers can be reasonably certain that the wheat is what it looks like or what it claims to be (Dixon & CIMMYT, 2009).

However, the concept of wheat quality is subjective, varying along the different actors in the wheat value chain, including milling, processing, end use, and nutritional quality (Guzmán et al., 2022). Therefore, the components of wheat grain, together with the physicochemical properties of wheat flour, play a decisive role in describing the grain quality characteristics (Pasha et al., 2010). The most important traits related with end-use quality being grain hardness, protein content, and color (Khalid et al., 2023; Zingale et al., 2023).

Grain hardness is an important factor in determining wheat quality, with the transition from common to durum wheat exerting a more pronounced impact on quality compared to alterations in protein quality or quantity (Manes, 2006). In bread wheat, the texture or hardness of the grain is determined by the mobilization of its components within the endosperm cells (Peña et al., 2002). Based on this

characteristic, bread wheat is classified as very hard, hard, or soft (Guzman et al., 2022).

Additionally, the color of the flour is also an important factor. The yellow pigment of durum wheat plays a crucial role in attracting consumers to products such as pasta (Beleggia et al., 2010). The yellow color in wheat flour is mainly due to the presence of carotenoids and anthocyanins, but also protein, starch and minor components (Sissons, 2008). In addition to consumer preferences, these compounds have a substantial impact on the nutritional value of wheat flour. They not only increase the visual appeal of wheat-derived foods, but also improve their antioxidant properties, which may lower the risk of specific diseases (Ficco et al., 2014; Padhy et al., 2022; Saini et al., 2020).

Proteins also play a crucial role in end-use quality. The protein content of the grains is usually between 7% and 18%. A large proportion of these proteins (around 80%) are prolamins, the proteins that make up gluten (Guzman et al., 2022). In the bread industry, gluten gives the dough viscoelasticity and flexibility, which allows it to bind the carbon dioxide from the fermentation process. Furthermore, in pasta production, it gives the dough the cohesion it needs to be moulded to the right shape (Brites et al., 2018). Therefore, the viscoelastic properties of gluten play a decisive role in the quality of wheat products (Shewry, 2019).

Wheat quality understanding is derived from the assessment of various quality attributes, which can provide valuable insights for bakers and manufacturers (Morris, 2015). Reliable methods for measuring flour quality are therefore essential, as they can facilitate informed decision making, process optimisation, and innovation. A variety of different small-scale tests and non-destructive methods have been explored for assessing wheat quality (Guzman et al., 2022). This originates in the context of wheat breeding programs, characterised by the creation of numerous crosses to introduce desirable traits, resulting in the generation of large populations for evaluation in subsequent breeding cycles. When managing this substantial volume of material, there is a growing reliance on high-throughput, small-scale tests to distinguish between acceptable and unacceptable/borderline samples, thereby optimizing the selection process.

Among the different available tests, the SDS sedimentation test (Sodium dodecyl sulphate sedimentation test) is one of the preferred. It is a physicochemical method for evaluating protein fractions, widely used to determine gluten

properties from small sample of flour (AACC International Approved Method 56–70). In addition, the version developed by (Dick & Quick, 1983) requires only 1g of flour. The SDSs test has been shown to be highly robust and reproducible, with the ability to delineate hexaploid wheat samples based on protein quantity and quality (Morris, 2007) . Moreover, the test has also been shown to be a reliable screening procedure for assessing gluten strength in durum wheat, with the ability to differentiate over a narrow range (Dexter et al., 1980).

Another widely used option in breeding programs is near-infrared spectroscopy (NIR), that enables non-invasive evaluation of grain samples or flour (Guzman et al., 2022), being suitable for both milled flour and wholemeal samples. The NIR is commonly used to assess protein and moisture content, from around 1g of flour (Peña et al., 2002).

Regarding procedures that focus not only on the proteins, the solvent retention capacity (SRC) constitutes an easy-to-perform small scale method for assessing quality, for which micromethods have already been established (Bettge et al., 2002; Guzman et al., 2015). This method allows for the estimation of water retention, degree of damaged starch, and the concentration of arabinoxylans, among other, from just 0,2g of whole flour if the micromethod is used (Guzman et al., 2015)

In addition to the methods described above, several other small-scale techniques are used extensively to evaluate wheat quality. Table 1 summarises these techniques and their specific applications.

Table 1: Small-scale methods for estimating wheat quality.

Test	Principle	Time	Sample	Characteristics	Application
GlutoPeak	Aggregation kinetics of gluten proteins	5 to 10 min	9 -10 g	- Low influence of the analyst - Few available studies	Bread & Durum wheat
Gluten Index	Gluten ability to pass through a sieve after centrifugation	10 min	10 g	Need to extract gluten - Overestimation of the value in case of low protein content samples - Low capacity of discriminating semolina of medium quality	Bread & Durum wheat
Glutograph	Gluten resistance to stretching	20 min	10 g	Need to extract gluten - High variability	Bread wheat
Mixograph	Measures flour water absorption and dough mixing characteristics	8 to 10 min	10-35g	-Optimum mixing time, gluten strength	Bread & Durum wheat
Mixolab	Dough resistance to both mechanical and thermal stress	45 min	50 g	Low influence of the analyst - Difficulty in following the set temperature profile	Bread wheat
Farinograph	Measures flour water absorption and dough strength	20 to 30 min	50 or 300 g	-Determination of dough properties	Bread & Durum wheat
Alveograph	Dough resistance to three-dimensional extension	50 min	250 g	- High influence of the analyst - Widely used in the field,	Bread & Durum wheat

Source: Cecchini et al., (2021); Guzman et al., (2022)

1.3.Wheat seed composition

The chemical composition of wheat grains is relatively consistent, with proteins, lipids, minerals, and starch (Goesaert et al., 2005; Shewry & Hey, 2015; Wieser et al., 2020a).

Lipids constitute a minor fraction of wheat grain, approximately 2-4%. The germ accounts for roughly two thirds (66%) of these lipids, while 15% reside in the bran, predominantly in the aleuronic layer. The remaining 20% are dispersed within the endosperm, partially encapsulated within starch granules (Carcea, 2021).

Minerals in wheat vary significantly between different cultivars, making wheat a notable source of essential nutrients (Choi et al., 2013). Minerals make up about 2.5%, of the endosperm, and include microelements such as zinc, iron, selenium, and magnesium (Javid Iqbal et al., 2022).

Starch constitutes the main component of the carbohydrate fraction, representing 65-75% of the dry weight. Variations in the distribution of wheat starch particle sizes and the amount of damaged starch granules are known to significantly affect baking performance (Hoseney, 1971).

Wheat grain protein content is around 12% (Diekmann, 2009; Shewry & Halford, 2002). These proteins are recognized as the pivotal factor in the breadmaking process (Morrison, 1988). The quantity and types (alleles) of proteins key for end-use quality (Johansson, 1996; Wieser et al., 2023a, b).

1.3.1. Endosperm proteins and their relation with quality

Proteins present in wheat kernels are classified into different groups, comprising functional proteins such as albumins and globulins, as well as storage proteins (Figure 3) (Day et al., 2006; Tosi et al., 2009). Grain storage proteins, also called prolamins, comprise approximately 70% to 80% of the total grain proteins and can be classified in gliadins and glutenins, which together form the gluten network. Prolamins in wheat exhibit significant variability, as highlighted by Shewry et al. (2001). Furthermore, they have unique chemical and physical properties that are fundamental to the distinctiveness of wheat flour (Wieser et al., 2023b).

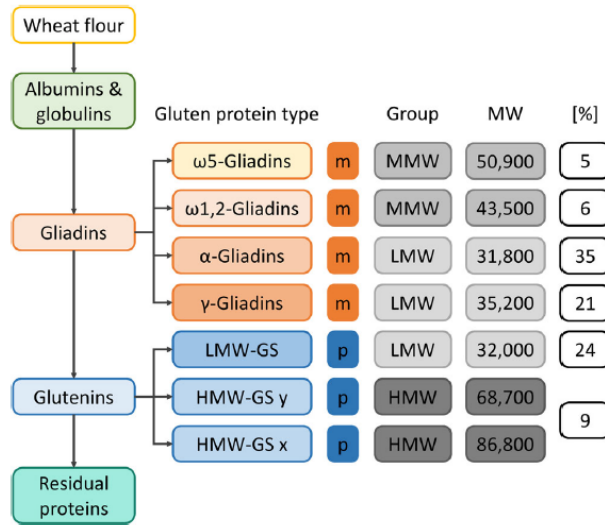


Figure 3: Classification of wheat proteins into different fractions and types

Source: reported by Wieser et al., 2023b.

Note: GS; glutenin subunits, HMW; high molecular weight, LMW; low molecular weight m; monomeric, MMW; medium molecular weight, MW; molecular weight, p; polymeric.

1.3.1.1. Importance of prolamins

Wheat prolamins represent a complex array of 71-78 proteins and play a pivotal role in determining the unique viscoelastic properties observed in wheat dough (Mejías et al., 2014). Prolamins can be classified into glutenins which form extensive macropolymers and monomeric gliadins. The specific alleles and the amounts of glutenins and gliadins in wheat significantly affect wheat flour end-use quality (Liu et al., 2023; Sharma et al., 2020).

Glutenins constitute approximately 45% of the total protein (Khalid et al., 2023) and are classified in high molecular weight glutenin subunits (HMW-Gs) and low molecular weight glutenin subunits (LMW-GS) (Payne, 1987). Glutenins are essential to shape the viscoelastic properties of wheat dough, thus significantly contributing to its strength and elasticity (Nunes-Miranda et al., 2017). Despite their relatively low abundance, they exert a significant influence on the technological and nutritional quality of products derived from wheat (Ferrante et al., 2006).

HMW-GS, which represent approximately 7-15% of the total wheat protein content (Wieser et al., 2023b), exhibit molecular weights ranging from 97 to 130 kDa (Jurkovic et al., 2000). HMW-Gs can be classified into X and Y subunits, which

include a signal peptide, an N-terminal domain, a repetitive domain, and a C-terminal domain. Differences among HMW-GS are generally due to variations in the length of the repetitive domain that correlates with allelic diversity (Shewry et al., 1995). The genes responsible for encoding HMW-GS are located on the short arm of homoeologous group 1, specifically at the *GLU-A1*, *GLU-B1* and *GLU-D1* loci. Within each of these loci, two closely linked genes are found, each encoding either an X- or a Y-type HMW-GS. However, it should be noted that the expression of these genes is not consistent, with most wheat bread cultivars typically expressing only 3-5 HMW-GS genes. Specifically, at the *GLU-A1* locus, the gene encoding the Y-type HMW-GS is consistently suppressed, while the gene for the X-type may or may not be expressed. At the *GLU-B1* locus, the expression of the gene encoding the Y-type HMW-GS may vary, while the X-type gene is consistently active. Finally, at the *GLU-D1* locus, both HMW-GS genes are consistently expressed (Shewry et al., 2003).

LMW-GS constitute approximately 20–35% of gluten protein (Labuschagne & Igrejas, 2020). That is, approximately one-third of the total seed protein and approximately 60% of the total glutenin content (Khalid et al., 2023). Their protein subunits have molecular weights ranging from 30 to 50 kDa (Liu, 1995; Pogna et al., 1990) and share a common primary structure consisting of a 20 amino acid signal peptide and a 13 amino acid N-terminal domain, which is absent in the LMW-I type (D'Ovidio & Masci, 2004). LMW-GS contain eight cysteine residues, the first and seventh cysteines typically involved in intermolecular disulphide bonds, while the rest participate in intramolecular disulphide bonds, essential for proper protein folding (Markgren et al., 2020). LMW-GS are classified into B, C and D type based on their mobility in SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) and isoelectric point (Jackson et al., 1983). Most LMW-GS are B type and are the key ones influencing the quality of durum wheat for pasta production (Sissons, 2008; Wrigley & Bekes, 2006). The genes encoding LMW-GS are located at the *GLU-2* and *GLU-3* loci on the short arm of homoeologous group 1. *GLU-A3*, *GLU-B3*, and *GLU-D3* are closely associated with *GLI-1* loci (Liu, 1995; Pogna et al., 1990; Singh & Shepherd, 1988). The number of LMW-GS genes varies between wheat varieties, with individual varieties expressing more than 15 genes, distributed as 4 to 6 at the *GLU-A3* locus, 3 to 5 at the *GLU-B3* locus, and 8 at the *GLU-D3* locus (Zhang et al., 2013). These subunits affect dough strength, mixing properties, and extensibility, with variations at the *GLU-A3* locus exerting the most substantial influence in bread wheat (Ibba, 2017).

Gliadins make up about half of the proteins that form gluten. The impact of gliadins on the quality of end-use wheat remains ambiguous. Extensive research has investigated the influence of gliadin composition on technological properties, revealing a general association between higher gliadin content and weaker gluten, leading to increased viscosity (Branlard & Metakovsky, 2006). Gliadins are monomeric proteins with molecular weights ranging between 30 and 80 kDa. They form intermolecular bonds facilitated by hydrogen bonds and hydrophobic interactions (Shewry et al., 2003). Gliadins are classified into distinct groups according to their electrophoretic mobility in A-PAGE (acid-polyacrylamide gel electrophoresis), into ω -, γ -, β -, and α -gliadins. Each group is encoded by genes located on the short arm of homoeologous chromosomes 1 and 6. The *GLI-1* loci within chromosome group homeologues 1 (*GLI-A1*, *GLI-B1*, and *GLI-D1*) predominantly control the expression of γ - and ω -gliadins, while the *GLI-2* loci (*GLI-A2*, *GLI-B2* and *GLI-D2*) within homeolog group 6 codes for α/β -gliadins (Metakovsky et al., 1984; Payne et al., 1982; Wrigley & Bekes, 2006). Notably, ω -gliadins lack cysteine residues, while α/β -gliadins and γ -gliadins possess 6 and 8 cysteine residues, which facilitates the formation of intramolecular disulphide bridges. Certain mutations enable α/β and γ -gliadins to contain an odd number of cysteines, allowing them to participate in intermolecular bonding within gluten, thus acting as polymerization terminators (Barak et al., 2015).

1.3.1.2. Genotyping and /or quantification of wheat endosperm proteins

The determination of prolamins alleles is crucial to understand genetic diversity and variation within wheat populations and to predict wheat quality (Chacón et al., 2020; Rodríguez-Quijano et al., 2010). This knowledge is particularly important for breeding programs that aim to improve the nutritional and technological properties of wheat (Nigro et al., 2020).

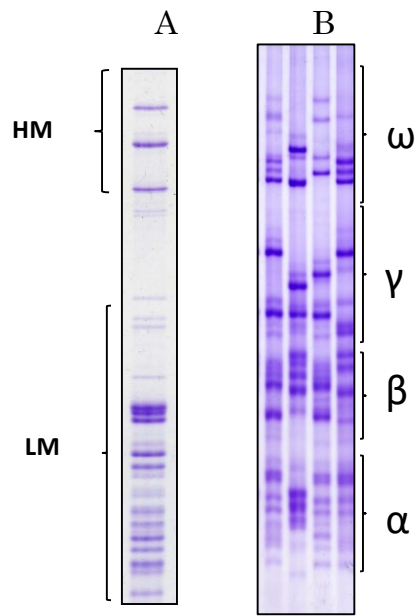


Figure 4: A. SDS-PAGE showing HMW-GS and LMW-GS. B. A-PAGE showing the different gliadins.

SDS-PAGE (Figure 4A) is the most used method for genotyping glutenins in wheat, as it allows for the precise identification of the different HMW-GS and LMW-GS with the aid of suitable controls (Liu et al., 2010; Wieser et al., 2023b). A-PAGE (Figure 4B) is the main protocol when gliadins are genotyped (Metakovsky & Novoselskaya, 1991). It has also been instrumental in assessing the genetic diversity of wheat lines, with considerable variation observed even among lines with the same parents (Metakovsky et al., 2020). Furthermore, the method has been used for the identification of wheat cultivars (Metakovsky et al., 2024), with the potential to enhance breeding programs and develop wheat strains with optimized protein profiles (Bushuk & Zillman, 1978).

In addition to the specific prolamin alleles, its quantification is also crucial to assess the nutritional and functional qualities of wheat, which directly impacts its use in food production and breeding programs (Wieser et al. 2023b). Various analytical methods have been developed to accurately assess wheat protein content, and high-performance liquid chromatography (HPLC) has emerged as a cornerstone technique in this domain. Reverse-phase HPLC (RP-HPLC) and size-exclusion HPLC (SE-HPLC), provide detailed insights into the composition and characteristics of prolamins (Labuschagne & Aucamp, 2004). RP-HPLC has been shown to accurately quantify different prolamin fractions, making it a valuable

method to estimate the HMW-GS to LMW-GS ratio and the gliadins to glutenins ratio (Gojković Cvjetković et al., 2019). SE-HPLC can be used to determine the percentage of UPP (unextractable polymeric protein in 0.5% DTT) which contains a higher proportion of GMP (glutenin macropolymer) than its extractable counterpart, thus providing some prediction of baking performance (Gupta et al., 1993). Autran (1994) also emphasized the importance of SE-HPLC in assessing the size range of gluten proteins and determining the proportions of aggregating and monomeric proteins in wheat.

1.3.2. Semolina and pasta color. Importance in durum wheat and methods for its estimation

The yellow-amber color of semolina has long been a key quality attribute for durum wheat-derived products, particularly for items such as pasta and semolina (Ficco et al., 2014). This trait plays an important role in shaping consumer preferences and determines the market value of durum wheat (Sarkar & Fu, 2022). The yellow color is the result of carotenoid pigment accumulation in the whole kernel and is measured in typically as the yellow index (YI) in semolina (Ficco et al., 2014). Consumers tend to prefer semolina and pasta with a bright yellow appearance, which also contains more nutritional value, as carotenoids exhibit antioxidant properties (Nishino et al., 2009). Therefore, developing durum wheat varieties with higher carotenoids content is a significant focus in breeding programs (Ficco et al., 2014).

A diverse set of carotenoids have been identified in wheat kernels, including lutein, β -carotene, zeaxanthin, β -cryptoxanthin and antheraxanthin (Lachman et al., 2017). Lutein is the predominant carotenoid, accounting for 86% to 94% of the total carotenoid content (Abdel-Aal et al., 2007). This molecule is evenly distributed across various grain layers, including aleurone and starchy endosperm (Borrelli et al., 2008). Lutein also exhibits greater stability during cooking compared to other commonly found carotenoids (Kean et al., 2011).

The first step in the carotenoid biosynthesis pathway involves the condensation of two molecules of geranylgeranyl pyrophosphate to form phytoene, a process catalysed by phytoene synthase (PSY) (Figure 5). Following this, a series of cascade reactions progressively occur. This biosynthesis pathway contains at least three rate-limiting steps: (i) during the early stage of phytoene synthesis, (ii) lycopene cyclization, and (iii) carotene hydroxylation. In wheat, the reaction

catalysed by PSY has been identified as the rate-limiting step, suggesting that it plays a crucial role in regulating carotenoid accumulation (Cong et al., 2009)

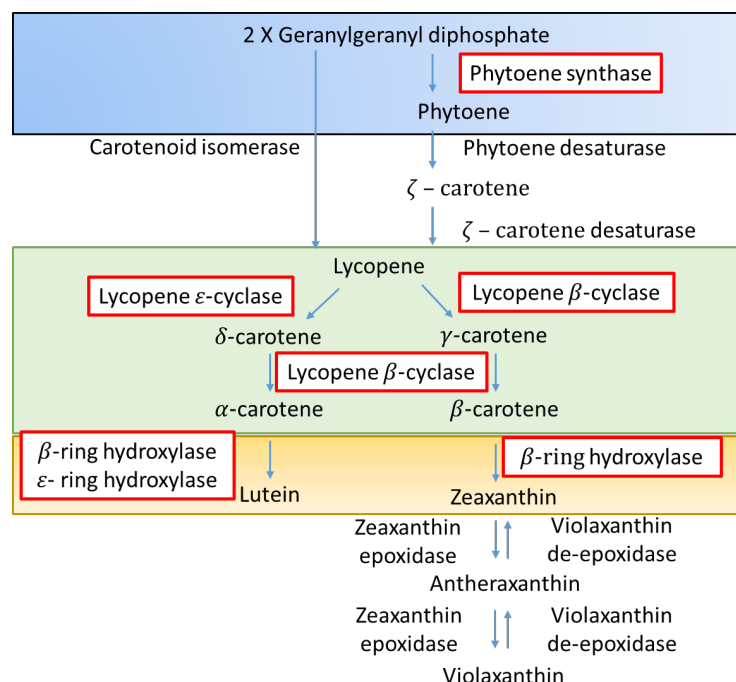


Figure 5: Carotenoid biosynthesis pathway

Note: The colored boxes are the three key limiting key steps in the carotenoid metabolism by Ficco et al., (2014).

The yellowness of durum wheat grains and their end products is influenced not only by carotenoid biosynthesis within the grain but also by carotenoid degradation during processing. This degradation is mainly attributed to oxidative enzymes, which contribute to discoloration and darkening during pasta production (Feillet et al., 2000; Hessler et al., 2002). A key family of enzymes responsible for this yellow color degradation are lipoxygenases (LOX) (Verlotta et al., 2010). LOXs are enzymes with dioxygenase activity that catalyse the specific oxygenation of polyunsaturated fatty acids containing 1,4-cis pentadiene structures, resulting in the formation of hydroperoxides. Radicals produced during the intermediate stages of linoleate hydroperoxidation can oxidize carotenoid pigments, leading to a reduction in the yellow color of pasta products (Siedow, 1991). However, the LOX reaction can be inhibited by carotenoid compounds, and the extent of carotenoid loss during pasta processing is said to be inversely correlated with the initial carotenoid content in semolina (Trono et al., 1999).

Various methodologies have been developed to quantify both the total pigment concentration and the specific carotenoid compounds present (Colasuonno et al., 2019). The flour color can be assessed using the yellow index (YI), which is determined by measuring the light reflectance from flour. For this procedure, the Minolta CR-300 Chroma Meter (Konica Minolta Pty Ltd, Macquarie Park, NSW), equipped with a pulsed xenon arc lamp, is the most widely used instrument. Provides measurements of the brown and yellow indexes after calibration with standard flour samples. Measurements include L^* (lightness, ranging from 0 for complete darkness to 100 for total brightness), a^* (red-green chromaticity) and b^* (yellow-blue chromaticity). The b^* value is directly related to lutein and carotene levels, which determine the variation in yellow intensity (Rodriguez-Amaya, 2004). Other reference methods for determining the total yellow pigment content (YPC) include, Standard Method 152 (ICC, 1990) of the International Association for Cereal Science and Technology (ICC) and the AACC International Official Method 14-50.01 (AACC International, 2013). Both methods involve extracting total pigments using water-saturated n-butanol, followed by spectrophotometric quantification of absorbance at 435.8 nm, the wavelength corresponding to the maximum absorption of lutein, the predominant carotenoid in durum wheat, according to US / Canada standards (Fu et al., 2017).

1.4. Genetics of end-use quality traits of durum wheat

Genetics plays a central role in determining key quality characteristics such as protein content, gluten strength, and flour color, which directly impact the quality of the final product, including pasta and semolina (Guzman et al., 2016). Understanding the genetic basis of durum wheat quality traits is crucial for making targeted improvements in breeding programs. Molecular markers, particularly Marker-Assisted Selection (MAS), have revolutionized the breeding process by allowing breeders to identify and select specific genetic traits more efficiently (Collard & Mackill, 2008). MAS enables the precise identification of allele variants in genes or Quantitative Trait Loci (QTL) responsible for desirable traits, reducing the time required to develop new wheat varieties (Laido et al., 2013). This approach also allows the combination of multiple desirable traits in a single breeding cycle, leading to significant improvements in durum wheat quality and yield (Collard & Mackill, 2008). However, to successfully conduct MAS it is necessary; i) to identify the genomic regions controlling the target trait, in which

a molecular marker will be developed, ii) identify superior alleles, and iii) estimate allele effects and genetic interactions between genes.

1.4.1. Genetic factors influencing durum wheat quality

1.4.1.1. Prolamin allelic variation and relation with quality

Variations in end-use quality related to gluten physicochemical characteristics are largely influenced by allele variations of endosperm storage proteins (Sissons 2008, 2022; Carrillo et al., 1990; Porceddu et al., 1998a; Turchetta et al., 1995). These proteins, classified as prolamins, are encoded by several complex and highly polymorphic loci, located on homoeologous groups 1 (glutenins and gliadins) and 6 (gliadins), in durum and bread wheat (Figure 6) (Payne et al., 19; 1981; Wrigley et al., 2006; Sissons, 2008; Shepherd, 1973).

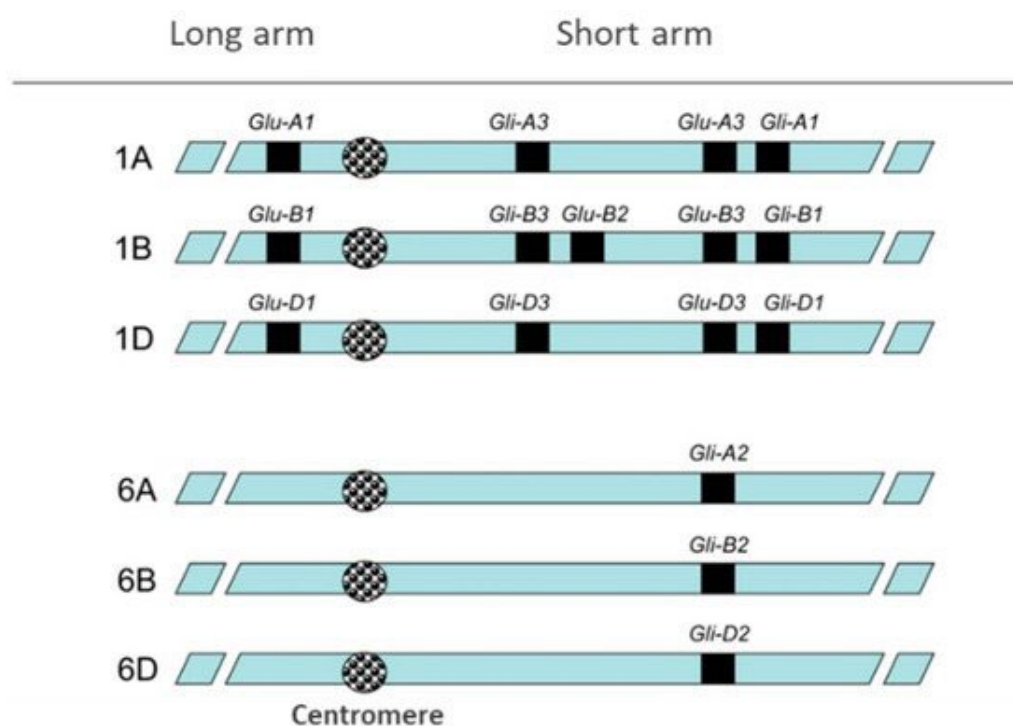


Figure 6: Location of glutenin and gliadin coding loci, in bread wheat

Note: For A and B chromosomes, locations are similar in durum wheat.

Source: Adapted from Garcia-Molina et al., 2019.

In durum wheat, the *GLU-1* loci encode the HMW-GS, and are located on the long arms of chromosomes 1A (*GLU-A1* locus) and 1B (*GLU-B1* locus), at 30 cM from the centromere. These loci are also present in bread wheat and have been extensively studied (Figure 6). Each of them contains two closely linked genes, *Glu-1-1* and *Glu-1-2*, which produce proteins classified as 'x-type' and 'y-type' based on differences in their molecular weights and isoelectric points (Payne et al., 1981).

For *GLU-A1*, more than 50 different alleles have been discovered in *Triticum* spp., the effects of some of them remaining controversial (Andrade et al., 2024). The most frequent alleles are *Glu-A1a* (subunit 1), *Glu-A1b* (subunit 2 *), and *Glu-B1c* (subunit null). Most studies agree that the *Glu-A1c* allele negatively influences gluten strength (Ciaffi et al., 1991; du Cros, 1987; Kaan et al., 1993; Porceddu et al., 1998b; Turchetta et al., 1995), since wheats carrying this allele present lower gluten strength than those with *Glu-A1a* and *Glu-A1b* (Brites & Carrillo, 2001; Oak et al., 2004; Raciti et al., 2003; Ruiz & Carrillo, 1995). Furthermore, Oak et al. (2004) found that allele *Glu-A1b* was linked to stronger gluten compared to allele *Glu-A1a*.

The *GLU-B1* locus is more relevant for gluten strength than *GLU-A1* according to Boggini & Pogna (1989). This locus is even more polymorphic than *GLU-A1*, and different studies have assigned different performance to the same *GLU-B1* alleles. While several studies have agreed that allele *Glu-B1e* (subunits 20x + 20y) is associated with a lower gluten strength compared to allele *Glu-B1b* (subunits 7 + 8), *Glu-B1d* (subunits 6 + 8), *Glu-B1h* (subunits 14 + 15) and *Glu-B1z* (subunits 7 + 15) (Babay et al., 2015; Boggini & Pogna, 1989; Brites & Carrillo, 2001; Magallanes-Lopez et al., 2017; Oak et al., 2004; Raciti et al., 2003; Ruiz & Carrillo, 1995; Trad et al., 2014), Nazco et al. (2014) reported a positive effect of allele *Glu-B1e* on SDSs test values. Other alleles that are considered to negatively affect gluten strength are the allele *Glu-B1a* (subunit 7), and *Glu-B1f* (subunits 13 + 16) (Babay et al., 2015; Oak et al., 2004). However, Magallanes-Lopez et al. (2017) found that allele *Glu-B1f* is associated with the strongest gluten in the genotypes analysed.

In durum wheat, LMW-Gs are considered more significant contributors to end-use quality than HMW-Gs (Ruiz & Giraldo, 2021), accounting between 55% and 27% of the variation in gluten index, respectively (Sissons et al., 2005). LMW-Gs (B-type) are coded by the loci *GLU-A3*, *GLU-B3*, and *GLU-B2* loci (Ruiz & Carrillo, 1993; Singh & Shepherd, 1988), being *GLU-A3* and *GLU-B3* loci closely linked to *GLI-A1* and *GLI-B1* (Singh & Shepherd, 1988) (Figure 6).

Various studies indicate that *GLU-A3* alleles have a limited impact on gluten strength (Aguiriano et al., 2009; Ruiz M& Carrillo., 1995; Vázquez et al., 1996). Actually, although Magallanes-Lopez et al. (2017) found a significant effect of *GLU-A3* alleles on gluten quality, this influence is still significantly lower than that of environmental factors and alleles at *GLU-B3*. Regarding the effects of the different alleles, studies are controversial, as for HMW-GS. The positive impact attributed to the *Glu-A3j* allele (subunit 6) is in conflict with the findings from Martinez et al. (2005) and Aguiriano et al. (2009), who associated this allele with weaker gluten. Other studies have elucidated the effects of different alleles, Aguiriano et al. (2009) reported that the *Glu-A3new1* allele (subunits 5* + 11 + 20) has a positive effect compared to the *Glu-A3e* allele (subunit 11), and Sissons et al. (2005) recommended avoiding the *Glu-A3l* allele when high gluten strength is desired.

At the *GLU-B2* locus, three alleles have been characterized with controversial results. Allele *Glu-B2a* (coding for subunit 12), allele *Glu-B2b* (a null subunit), and allele *Glu-B2c* (coding for subunit 12*). Studies have shown no significant differences between the alleles *Glu-B2a* and *Glu-B2b* (Babay et al., 2015; Brites & Carrillo, 2001; Magallanes-Lopez et al., 2017; Martinez et al., 2005; Ruiz & Carrillo, 1996). However, other research suggests that allele *Glu-B2a* is associated with stronger gluten compared to allele *Glu-B2b* and allele *Glu-B2c* (Aguiriano et al., 2009; Martinez et al., 2005; Nazco et al., 2014). In contrast, Sissons et al. (2005) reported that cultivars carrying the *Glu-B2a* allele exhibited weaker gluten properties.

Finally, numerous studies have shown that allele variants at *GLU-B3* exert a significantly greater influence on gluten quality compared to variants in *GLU-A3* and *GLU-B2* (Brites & Carrillo, 2001; Magallanes-Lopez et al., 2017; Martinez et al., 2005; Ruiz & Carrillo, 1995; Vazquez et al., 1996). This locus is located on the short arm of chromosome 1B, between *GLI-B1*, at 2 cM, and the centromere (Figure 6) (Pogna et al., 1990). As previously described for the other LMW-Gs coding loci, some discrepancies have been observed in the *GLU-B3* allele rankings. For example, Magallanes-López et al. (2017) found that the allele *Glu-B3r* (subunits 2 + 4 + 15 + 19) was associated with better values than the allele *Glu-B3t* (subunits 2 + 4 + 14 + 15 + 19). This contrasts with the findings of Brites & Carrillo (2001), who reported that allele *Glu-B3r* exhibited lower values than both alleles *Glu-B3t* and *Glu-B3j* (subunits 4 + 6* + 15 + 19). Furthermore, (Sissons et

al., 2005) indicated that the allele *Glu-B3w* (subunits: 2 + 4 + 15 + 17) negatively affected gluten strength.

In summary, both the HMW-GS and LMW-GS loci affect durum wheat, although LMW-GS show a greater effect. Regarding the better alleles to select in breeding programs, results remain controversial, probably due to environmental or epistatic effects.

1.4.1.2. Genes associated with durum wheat color.

The inheritance of yellow pigment (YP) is known to be multifaced and predominantly controlled by additive gene action (Elouafi et al., 2001; Johnston et al., 1983; Mares & Campbell, 2001). Moreover, it has been shown to have a high heritability in various studies (Clarke et al., 2006; Elouafi et al., 2001; Parker et al., 1998; Patil et al., 2008). The main gene that significantly influences yellow pigment content (YPC) and yellow index (YI) is *PSY*, which is crucial for the accumulation of carotenoid in the kernels (Gallagher et al., 2004). Phylogenetic analyses have revealed three distinct *PSY* isoforms: *PSY1*, *PSY2* and *PSY3*, which are mapped to homoeologous chromosome groups 7, 5, and 3, respectively (Dibari et al., 2012). The loci most extensively studied have been *PSY1-A1* (located at 7AL), *PSY1-B1* (located at 7BL), *PSY2-A1* (located at 5A) and *PSY2-B1* (located at 5B) (Howitt et al., 2009). The first study to demonstrate that the *PSY1* gene is located on chromosomes 7A and 7B in durum wheat was conducted by (Atienza et al., 2007). The gene coding for *PSY1-B1* was found to co-segregate with a carotenoid quantitative trait locus (QTL), while the *PSY1-A1* gene acted as a co-dominant marker, explaining 20–28% of the phenotypic variability (Pozniak et al., 2007).

Actually, quantitative trait loci (QTL) mapped to chromosomes 7A and 7B have been shown to account for more than 50% of observed phenotypic variability (Elouafi et al., 2001; Parker et al., 1998; Zhang et al., 2008). Therefore, most mapping studies agree that group 7 chromosomes play a significant role in influencing grain pigment in durum wheat (Atienza et al., 2007; Elouafi et al., 2001; Parker et al., 1998; Patil et al., 2008; Pozniak et al., 2007; Zhang & Dubcovsky, 2008).

However, the influence of *PSY1* loci on the yellowness of wheat endosperm in durum wheat varies depending on genetic background (Colasuonno et al., 2019). Consequently, the percentage of explained variability in endosperm yellowness attributed to *PSY1-A1* has been reported to range from medium (10–30%) to high

(30–50%) and even very high (> 50%) (Blanco et al., 2011; Colasuonno et al., 2014). On the other hand, the variability explained by *PSY1-B1* has been documented to range from low (< 10%) to medium (10–30%) (Roncallo et al., 2012; Zhang & Dubcovsky, 2008). Accordingly, the influence of alternative *PSY-A1* alleles appears to be the most significant factor contributing to the variation in semolina yellowness (SY) between different populations of durum wheat (Campos et al., 2016; Vargas et al., 2016).

As noted above, the oxidative degradation of carotenoid pigments during pasta processing is primarily facilitated by the enzyme lipoxygenase (LOX). The wheat genes that encode the various LOX isoforms are called *LPX*.

LPX-1 and *LPX-3* encode the LOX-1 and LOX-2 isoforms, respectively, and are located on chromosome 4, while *LPX-2*, which encodes the LOX-3 isoform, is found on chromosome 5 (Garbus et al., 2009; Manna et al., 1998; Verlotta et al., 2010; Zhang & Dubcovsky, 2008). Among these, *LPX-1* transcripts are the most abundant in mature grain (De Simone et al., 2010), suggesting that the LOX-1 isoform may play a crucial role in the oxidation of carotenoid pigments during pasta processing. This hypothesis is further supported by evidence that points to the presence of a major QTL for total LOX activity on chromosome 4B, where three copies of the *LPX-1* gene, *LPX-B1.1*, *LPX-B1.2* and *LPX-B1.3*, along with at least three different *LPX-B1.1* alleles (*Lpx-B1.1a*, *Lpx-B1.1b* and *Lpx-B1.1c*), have been described (Hessler et al., 2002; Verlotta et al., 2010; Zhang et al., 2008). Research has shown that the deletion of *LPX-B1.1* is associated with a marked reduction in LOX activity in semolina (Verlotta et al., 2010). Therefore, selecting and incorporating this allele into breeding lines could significantly reduce pigment loss during pasta processing, ultimately improving both the aesthetic and nutritional qualities of pasta products.

1.4.2. Importance of Quantitative Trait Loci (QTL) analysis for quality traits

Durum wheat QTLs identification has focused on its main agronomic traits, such as, grain yield (Arriagada et al., 2022; Taranto et al., 2023b) or disease resistance (Viviani et al., 2025). Besides, QTL analysis has also been instrumental in identifying key genomic regions associated with wheat quality traits (Zingale et al., 2023). Although much of the focus has traditionally been on the glutenin subunits of high molecular weight (HMW) and low molecular weight (LMW),

recent studies have identified additional QTL outside of these regions that also significantly contribute to wheat quality (Johnson et al., 2019). These QTLs are related to traits such as dough strength, extensibility, grain hardness, and protein content. The discovery of these new regions highlights the complexity of wheat quality traits and emphasizes the need to expand our understanding of the effects of known alleles while identifying new genetic regions that influence these traits.

Furthermore, these findings underscore the importance of continuing to develop advanced genotyping techniques and molecular markers for more precise selection in breeding programs. By integrating QTL analysis with advanced genetic maps and leveraging new regions related to quality traits, researchers can improve the accuracy of marker-assisted selection (MAS). This will facilitate the development of durum wheat varieties with superior end-use quality, allowing fine-tuning of traits such as flour and pasta color, protein content, and gluten strength.

1.4.2.1. Available genetic maps and QTL studies.

QTLs identification depends on the availability and/or development of high-quality genetic maps. In durum wheat the first genetic map was developed in 1998 (Blanco et al., 1998), comprised 1352cM and included 213 molecular (RFLPs) and biochemical markers. Since then more than sixty maps including intra- and inter-specific maps have been developed (Colausuonno et al., 2021). The first maps were based on mainly on AFLPs, RFLPS, SSRs and biochemical markers. However, the DarT markers provided better coverage and were soon used to develop the first consensus maps (Maccaferri et al., 2014). In the last years with the development of SNPs platforms and DArTseq technology based on GBS (genotyping by sequencing), new highly saturated maps have been developed, allowing the development of a denser consensus genetic map (Maccaferri et al., 2015). That map integrated 13 data sets from independent biparental populations, included 30144 markers (including 26 626 SNPs and 791 SSRs) and spanned 2631 cM along all 14 durum wheat chromosomes. The last durum wheat maps based on high-throughput genotyping platforms typically include between 1000 to 10000 molecular markers, spam around 2500 cM, and have facilitated the discovering of new durum wheat QTLs (Colasuonno et al., 2021).

Regarding to quality traits, several studies have summarized the quality QTLs present in the durum wheat genome (Coalusonno et al. 2021; Kiszonas & Morris, 2018; Zingale et al., 2023). It must be noted that quality QTLs have been

identified in all the durum wheat chromosomes (Figure 7), according to Colasuonno et al. (2021) that summarized 15 QTL for quality traits detection studies, an identified a total of 171 QTLs. Most of the studies summarized by Colasuonno et al. (2021), focus on quality traits like yellow pigment content, gluten strength, grain protein content. While only a few studies have been developed about specific prolamins fractions contents, that might uncover key QTLs related to prolamins synthesis (Plessis et al., 2013; Zhou et al., 2021). Thus, genetic control of such important traits remains unexplored.

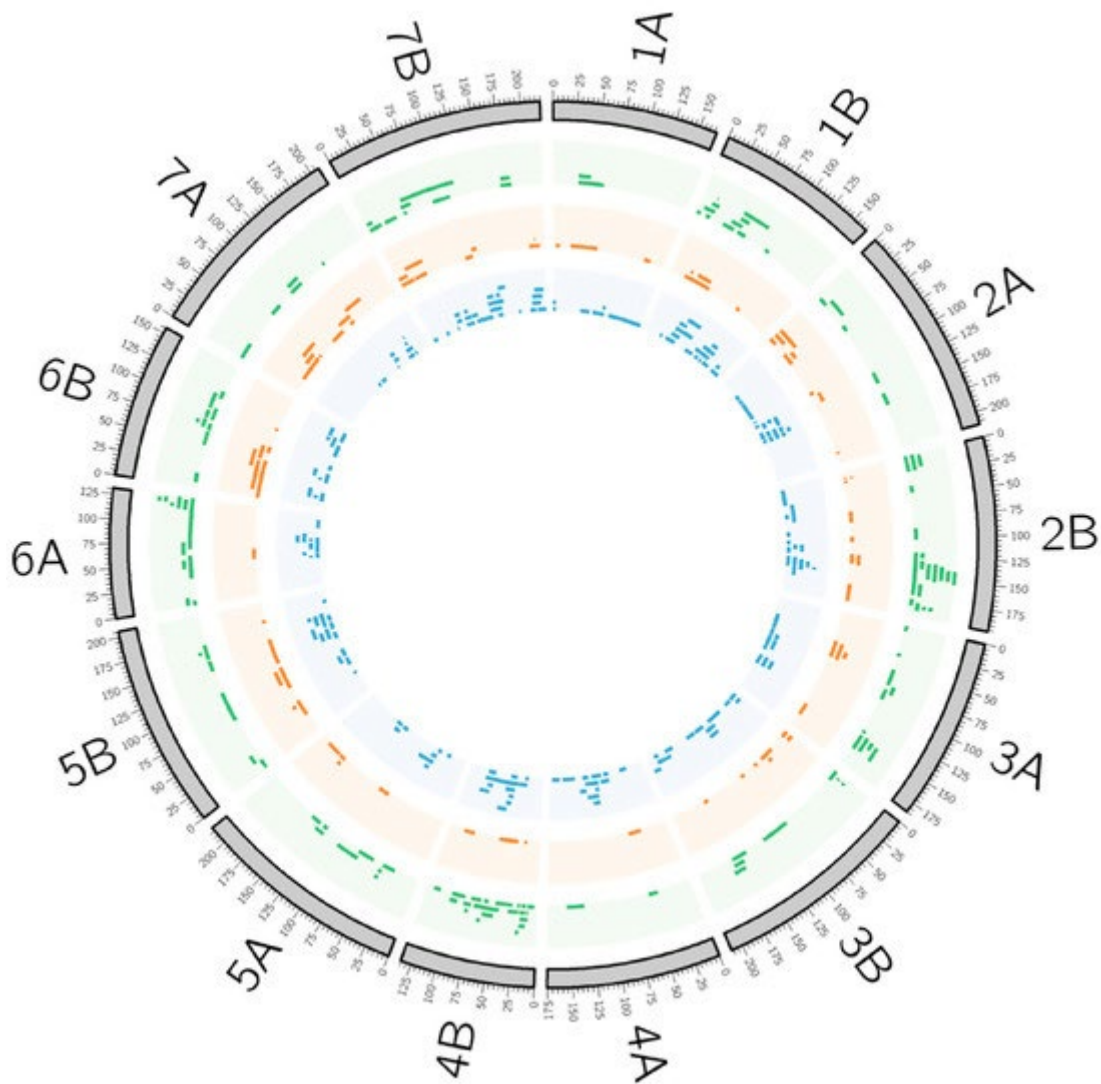


Figure 7: Quantitative trait loci (QTL) projection into the consensus map developed by Maccaferri et al. (2015).

Note: Color code: green: abiotic QTL, orange: biotic QTL, blue: quality QTL.

Source: Colousanno et al., 2021.

1.5.Objectives

The overall objective of this thesis project is to develop and implement methodologies to identify new genomic regions in wheat controlling bakery and semolina quality. In order to provide advanced tools for end-use-quality focussed breeding programs.

To successfully address this objective, the following partial objectives are defined:

1. To develop a fast method for genotyping and quantification of the different glutenins fractions.
2. To test the potential of the SDS-PAGE based method developed in bread wheat samples.
3. To develop a new genetic map in durum wheat and test its power to identify QTL related to yield and grain protein content.
4. To identify new QTLs related with key quality traits in durum wheat.
5. To investigate the effects of different *Lipoxygenase 1* and *Phytoene Synthase 1* alleles on durum wheat flour color.

2. State of the art

2.1. Image-assisted quantification of high and low molecular weight glutenin fractions in wheat by SDS-PAGE

The versatility of wheat flour is due to the unique functional properties of gluten, formed by endosperm proteins. Gluten has been studied for almost 300 years, however the exact structure, nature and formation of this complex network has yet to be fully understood revised by (Shewry & Belton, 2024). Nevertheless, several facts have already been established. Gluten is mainly formed by prolamins, that can be classified in monomeric gliadins, which confer mainly extensibility and viscosity to the dough, and polymeric glutenins, large polymers that contribute to dough strength and elasticity (Wieser et al., 2006). Monomeric gliadins, that account for around 50% of gluten proteins, are divided in four subfamilies according to their mobility in A-PAGE (acid polyacrylamide gel electrophoresis) (Woychick et al., 1961). On their turn, polymeric glutenins are classified in high molecular weight glutenins (HMW-GS) and low molecular weight glutenins (LMW-GS) based on their relative mobility in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Payne 1987). HMW-GS represent around the 7-15% of gluten proteins, while LMW-GS are more abundant representing between 20-35% of total prolamins (revised by Wieser et al., 2023b).

Gluten qualitative and quantitative composition greatly influence its properties, thus determining flour end-use. The gliadins to glutenins ratio directly impact gluten baking performance. For example, an increase in gliadin proportion will decrease peak viscosity (Li et al., 2020), and higher ratios will negatively affect bread volume and crumb firmness (Barak et al., 2013). Besides, the fact that glutenins form polymers that reach a molecular mass of more than 250kD in glutenin macropolymers (GMP) (Labuschange & Igrejas, 2020; Shewry & Belton, 2024) has to be considered. So, a shift towards higher molecular weights, corresponding to higher GMP content, results in a stronger dough with greater resistance to mixing and increased stability (Wieser et al., 2023b). GMP structure is determined by (1) allelic variation of HMW-GS, (2) proportion of terminators and (3) the ratio of HMW-GS to LMW-GS (Wieser et al., 2006).

HMW-GS allelic variation, even though being the less abundant prolamins, is acknowledge to be responsible of around 60% of observed variation in bread wheat

end-use quality (Gupta et al., 1993). Among the three homoeoloci coding for HMW-GS, *GLU-D1* is considered the most important (Wang et al., 2017). Actually, the allele *Glu-D1d* (5+10) is generally favored in breeding programs in bread wheat, as the extra cysteine residue of its derived glutenin subunit facilitates the formation of GMPs thus producing an increase in gluten strength (Zhao et al., 2020). Terminators are mainly gliadins with an odd number of cysteine residues and LMW thiol (Schmid et al., 2017). With respect to HMW to LMW-GS ratio, an increase in HMW-GS proportion shifts the proportion of GMP increasing their amount and also gluten strength (Wieser et al., 2023a). The HMW-GS alleles and terminators types are genetically determined (Cinco-Moroyoqui & MacRitchie, 2008). However, the expression level of glutenins loci, and thus the abundance and ratio of these proteins is more complex. Besides the genetic background, environmental and crop management factors, including irrigation, ambient CO₂, and fertilization, also play a role in HMW to LMW-GS ratio and contents in GMP recently reviewed by (Wieser et al., 2023b).

According to the stated above, qualitative and quantitative analysis of glutenins may allow the prediction of flour properties, and aid selection in wheat breeding programs aiming to develop wheat varieties targeted for specific industrial purposes. Since, thirty years, SE-HPLC (Size exclusion high performance liquid chromatography) can be used to determine the percentage of UPP (polymeric protein unextractable in 0.5% SDS) that contains a higher proportion of GPM than its extractable counterpart, thus providing some prediction of baking performance (Gupta et al., 1993). Besides, Marchylo et al. (1989) developed the RP-HPLC method (Reversed phase high performance liquid chromatography) that, later refined by Wieser et al. (1998), allows a precise estimation of HMW-GS, LMW-GS, gliadins and the ratio between different protein fractions. HPLC-based methods with technical modifications have been developed, and are still the most widely employed (Plessis et al., 2013; Ribeiro et al., 2015; Wang et al., 2017; Zhou et al., 2021; Haraszi et al., 2020; Wieser et al., 2023a). Nonetheless, their use for breeding programs is not as extensive as HMW-GS genotyping due to some major drawbacks: first, the lack of consistency over different labs, which is probably due to differences in set up, running as well as types of equipment, column and even temperature, that might also affect the results (Helguera et al., 2020); second, the complexity of the method and high cost associated with purchasing and maintaining HPLC equipment, that is far more expensive than that required to perform SDS-PAGE (Haraszi et al., 2020).

Simpler procedures have been developed or tested in order to facilitate prolamins quantification. For example, Suchy et al. (2007) set up a protocol using organic solvents to separate gliadins and glutenins, and later quantified them spectrophotometrically based on their absorbance at 280 nm. However, this method does not permit to estimate the HMW to LMW-GS ratio. The SDS-PAGE procedure has also been adapted for prolamins quantification. So, Dhaka and Khatkar (2015) & Koga et al. (2016) estimated HMW to LMW-GS ratios based on image analysis of electropherograms, finding interesting correlations. However, each of them employed their own specific protocol, significantly different from the methods developed to identify glutenin alleles. Even more, they did not contrasted the performance of their approach compared to RP-HPLC. This is of utmost importance, considering that Cinco-Moroyoqui & MacRitchie (2008) found inconsistencies when comparing different extraction protocols coupled with SE-HPLC, RP-HPLC and SDS-PAGE for quantification. After assessing different methodologies, these authors concluded that the difficulty of conducting reproducible SDS-PAGE experiments and the change of band intensity that might occur during destaining steps makes SDS-PAGE inappropriate for glutenins quantification.

Ideally, the development of a standard method that allows both accurate prolamins genotyping and calculation of HMW to LMW-GS ratio may make possible the estimation, by a single analytical procedure, of two of the main factors governing the formation of GMP (Wieser et al., 2006). It would particularly facilitate gluten strength prediction in early generations, in which not much grain is available. Recent advances in imaging and protein quantification by SDS-PAGE suggest that it might now be possible. Alonso Villela et al. (2020) presented a protocol of image analysis for electropherograms that allows the quantification of unknown bacterial proteins. Based on their approach, we have tested the suitability of the standard SDS-PAGE protocol for glutenin genotyping to estimate glutenins abundance and HMW to LMW glutenin subunit ratio. First, we characterized a biparental population, and determined the available diversity for glutenins at qualitative and quantitative levels by SDS-PAGE and RP-HPLC, respectively. Later, we selected a diverse subset and demonstrated the suitability of SDS-PAGE for glutenin quantification.

2.2. Impact of quantitative and qualitative Glutenin Variation on wheat quality within a similar genetic background

Wheat gluten has unique chemical and physical properties that are the basis for the wheat quality, determining the optimal performance of wheat-based products (e.g. bread, noodles, biscuits, etc.). Gluten is mainly composed of the storage proteins accumulated in the endosperm i.e. monomeric gliadins (prolamins) and polymeric glutenins (glutelins), which are the main responsible for its characteristic properties. It was found that the percentage of glutenins and gliadins improves as the total protein content of the wheat flour increases. However, the increase is more pronounced for glutenins as compared to gliadins. Also, the contents of glutenin subunits explain the variation of dough and gluten properties better than the contents of gliadins (Zhang et al., 2007). In particular, elasticity, as an important physical property of wheat dough, has been ascribed to the glutenin fraction (Wieser & Kieffer, 2001). Glutenins form polymers that reach a molecular mass of more than 250kD in glutenin macropolymers (GMP). So, a shift towards higher molecular weights, corresponding to higher GMP content, results in a stronger dough with greater resistance to mixing and increased stability (Wieser et al., 2023b).

Polymeric glutenins are classified as high molecular weight (HMW) and low molecular weight (LMW) glutenins based on their relative mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Payne et al., 1987). Both the quantity and composition of HMW glutenin subunits (HMW-GS) greatly influence gluten viscoelasticity by modifying the polymer size distribution and aggregative properties of gluten proteins (Popineau et al., 1994). Their contribution to gluten strength was more important compared with LMW-GS, despite accounting for only about 7%-15% of the total protein in flour. HMW-GS variability is genetically determined by the allelic variation at the homoeoloci *GLU-1* (Payne & Lawrence, 1983; McIntosh et al., 2013), which displays different effects on quality parameters (e.g. Payne et al., 1987). In general, the *GLU-D1* locus has the strongest effect on gluten strength, the allele *Glu-D1d* (1Dx5 + 1Dy10) having a more beneficial effect than other alleles as *Glu-D1a* (1Dx2 + 1Dy12) (e.g. Popineau et al., 1994; Lopez-Fernandez et al., 2021). Although *Glu-D1d* is generally favored in breeding programs in bread wheat, the proportion of *Glu-D1a* in wheat varieties is higher, especially in landraces (López-Fernández et al., 2021). On the other hand, some controversial results have been obtained

related to the effects of HMW glutenin alleles and quality, even for those varieties having *Glu-D1a* or *Glu-D1d* alleles (Dhaka & Khatkar, 2015). This could be due to positive or negative interactions with other quality-related subunits encoded at other loci. The genes coding for LMW-GS are located on the short arms of homoeologous group 1 chromosomes at the *GLU-A3*, *GLU-B3* and *GLU-D3* loci, and are tightly linked to ω -gliadins encoded at the *GLI-1* loci. Extensive variation was found at the three *GLU-3* loci, each exhibiting distinct allelic effects on gluten strength (Gianibelli et al., 2001; Liu et al., 2010).

Several studies have shown that gluten quality was also affected by quantitative differences in the HMW glutenin subunits (Singh et al., 1990; Popineau et al., 1994; Wieser & Kieffer, 2001; Zhang et al., 2007). For some allelic variants, positive effects were attributed to quantitative differences rather than qualitative ones (Singh et al., 1990). According to those findings, an increase in HMW-GS proportion relative to LMW-GS shifts the proportion of GMP, increasing their amount and also gluten strength (Wieser et al., 2023b). Although the expression level of glutenin loci is significantly affected by the genotype (Panozzo & Eagles, 2000), the analyses and thus the amount of HMW and LMW-Gs are more complex than the study of *GLU-1* allelic variation. In addition to genetic and environmental factors, genetic background also plays a role in HMW to LMW-GS ratio and contents in GMP (Wieser et al., 2023b).

Depending on the final product, different glutenin contents are required. Bread-making quality can be improved through the increase in the amount of total glutenin whereas a high quantity of protein and its fractions have a moderately undesirable effect on noodle processing quality (Zhang et al., 2007). Accordingly, qualitative and quantitative analysis of glutenins may allow the prediction of flour properties and aid selection in wheat breeding programs aimed at developing wheat varieties for specific industrial purposes. A large number of studies have examined the effects of glutenin alleles on wheat quality, but few of them analysed their relation with the quantity of glutenin fractions.

Recombinant inbred lines (RILs), near isogenic lines (NILs), doubled haploid lines (DHs) and biotypes of the same variety are ideal materials for analysing the HMW-GS effects on quality as they share a similar genetic background.

So, the objectives of the present study are: 1) to analyse the effects of different glutenin alleles (HMW-Gs and LMW-Gs) on gluten quality and on the quantity of glutenin fractions, and 2) to examine the relationship between gluten

quality and the quantity of glutenin fractions considering the glutenin allelic variation, using a set of intra-variety biotypes to diminish the effect of the genetic background. Although wheat quality can be assessed using various analytical methods, this study focused on two methods (GPC and SDSs) due to the limited availability of wheat grains.

2.3. A new genetic map in durum wheat, allows the detection of QTL related to yield and grain protein content

Wheat (*Triticum sps.*) is one of the major staple crops, providing about 20% of dietary calories and proteins (Shewry et al., 2015). The most cultivated species is bread wheat, however durum wheat (*Triticum turgidum* L. *ssp. Durum* 2n=28, genomes AABB), also have a major importance in countries located in the Mediterranean basin (Martinez-Moreno et al., 2022). Actually, to over 1 billion people worldwide living in poverty, durum wheat is the primary food source providing between 20 to 50% of calories and 20% of proteins daily (Grosse-Heilmann et al., 2024). Thus, developing cultivars with enhanced yield in durum wheat will have a worldwide impact, especially in several countries living in poverty.

Breeding in any species is highly enhanced if the genes controlling key traits and their allele effects are known. As, the identification of major regions in the genome controlling these traits, quantitative trait loci (QTL), offers the opportunity to track them with molecular markers through molecular-assisted selection (Colasuonno et al., 2021). Lately, advances in high throughput genotyping technologies offer the possibility to construct saturated genetic maps (Russo et al., 2014), therefore QTLs can be located with high resolution. Several genotyping tools are available for durum wheat, including SNPs arrays, such as, the Illumina Wheat 90K iSelect SNP genotyping array, or the Wheat 50K Triticum TraitBreed array among others reviewed by (Colasuonnno et al., 2021). However, SNPs arrays will only be useful, as long as, they include polymorphic markers for the cultivars or populations under study. In this context DArTseq technology based on genotyping by sequencing (GBS) (<https://www.diversityarrays.com/>) guaranties the identification of polymorphic markers, and has allowed Maccaferri et al. (2015) to construct a second-generation consensus map for durum wheat.

In wheat, yield is the key breeding trait, and can be dissected into three principal components, including number of spikes per area, grain number per spike and

grain size (Lopez-Fernandez et al., 2023). Among those traits grain size is considered the most heritable one. Grain size can be specified by grain length, grain width, grain aspect, grain area, and grain weight expressed as a 1000 - kernel weight (TKW) (Manni et al., 2025). Linkage mapping and genome - wide association study (GWAS) identified and mapped over 100 QTL for grain weight and size distributed across nearly all wheat chromosomes (Taranto et al., 2023a). Although, additional studies including non-previously screened variability, have the potential to identify new genes according to Malik et al. (2021). Besides yield, grain protein content and quality related traits like flour color have being also the focus of durum breeding and QTL detection (Colausonno et al., 2021, 2019).

The bi-parental population Endural X Aldura has been developed from two ancient varieties of durum wheat (Rubianes, 2007). Endural is variety developed by Benoist in 1989 (<http://wheatpedigree.net/sort/show/18341>) and Aldura an Italian durum wheat (K-61921, <http://wheatpedigree.net/>). This population is particularly interesting as previous analyses have shown the existence of transgressive lines in the population for end-use quality traits (Rubianes, 2007). However, it has never been characterized for grain size traits or flour color, nor it is available a high-saturated genetic map to aid in the identification of the genetic determinants of end-use quality traits.

The aim of this study was to develop a new genetic map in durum wheat, and to detect QTLs related with grain size, grain protein content and flour color. In order to do so, we have genotyped by DArTseq the population developed between the cultivars Endural and Aldura, constructed a highly saturated genetic map, and coupled this map with phenotypic characterization conducted along four different seasons to identify phenotype-genotype associations (QTL).

2.4. Identification of genetic factors controlling rheological properties in durum wheat by QTL detection

Durum wheat (*Triticum turgidum* L. *ssp. Durum* 2n=28, genomes AABB), is a globally minor crop, accounting for less than 7% of the worldwide wheat production. Whose production is concentrated in small areas where it can be considered a main cereal crop (Martinez-Moreno et al., 2022). Durum wheat presents a higher grain protein content, harder endosperm, yellower grain color and more extended shelf life respect to bread wheat (Sissons, 2008), all traits that

influence durum wheat end-use. This species is almost exclusively used for human consumption, with around 75% of the production employed to obtain pasta products (e.g., spaghetti, macaroni, etc.) (Grosse-Heilmann et al., 2024). But it also holds significance in various regions of Africa, the Middle East, and Central Asia for producing local cuisine, such as couscous, bulgur, frike, different types of flat breads and other semolina - based products (Peters Haugrud et al., 2023).

Due to its use for the human consumption, development of cultivars with better technological quality for industrial processing has been a main focus in durum wheat breeding programs during the XX century (Subira et al., 2014; Zingale et al., 2023). It is well known that, in pasta making, good quality depends on the formation of a continuous network of gluten to retain the gelatinized starch granules during pasta cooking (Ruiz & Giraldo, 2021). Previous studies have found that modern durum wheat cultivars present stronger gluten and have attributed this to major expression of B-type LMW-GS and higher glutenin/gliadin ratio reviewed by (Zingale et al., 2023). But it must be noted that, although weak and inelastic gluten is linked to poor pasta cooking quality, how much strength is optimal is not known (Sissons, 2008). Moreover, rheological properties also depend on starch (mainly as damaged starch) and arabinoxylans (pentosans), that also influence flour-water absorption (Guzman et al., 2015).

Several methodologies have been employed to characterize those traits, with fast micro-methods being the preferred for breeding programs (Sissons et al., 2004). SDSs allows a rapid estimation of gluten strength from just 1g of whole flour when the micro-method is used (Dick & Quick, 1983). While solvent retention capacity tests (from now on SRC) (AACC Methods 56-10 AWRC and 56-11 SRC) determine the capacity of flour to hold different solvents: water, associated with the overall water holding capacity of all flours constituents; 50 g/L sodium carbonate, related to the damaged starch content of the flour; 500 g/L sucrose, associated with the concentration of arabinoxylans; and 50 g/L lactic acid, associated with the glutenin swelling capacity and 0,1N sodium bicarbonate, inversely correlated with cookie spread. Finally, a newly developed method by Bouabdellah et al (2024, detailed in the results section 4.1) provides a reliable quantification of the different glutenin fractions.

Knowledge about the superior alleles for the traits influencing rheological properties is also key for breeding programs. Since the development of Payne score for bread wheat (Payne et al., 1987) it has been established that HMW-Gs alleles

predicts gluten strength. Regarding to durum wheat, LMW-GS are the largest contributor to gluten protein and functional properties, accounting for around the 80% of variation in SDSs test results reviewed by (Ruiz & Giraldo, 2021). Besides, gliadins are also considered a good predictor of durum wheat quality, with *Gli-B1* γ -45 preferred over *Gli-B1* γ -42 due to its linkage with superior LMW-GS alleles (Giraldo et al., 2020). Regarding to specific glutenin alleles, there are controversial results, as example *Glu-B3w*(2+4+15+17) reviewed by (Ruiz & Giraldo, 2021). The effects of prolamin alleles on quality can be evaluated in cultivar collections or more accurately using segregating populations such as recombinant inbred lines (RILs) reviewed by (Ruiz & Giraldo, 2021).

Besides prolamin coding loci, other genomic regions have been associated with prolamins or rheological behaviour in bread and durum wheat. In bread wheat, Storlie et al. (2009) demonstrated that there are on average 9 chromosome arms controlling HMW-Gs genes expression. Zhou et al. (2021) identified two main QTL clusters in chromosomes 6A and 6B associated with glutenin and glutenin fraction contents, and Plessis et al. (2013) identified 7 transcription factors inside genomic regions linked with glutenin and gliadin quantity. In durum wheat, apart from homoeologous group 1, QTLs for grain protein content have been found in all the chromosomes, for gluten strength in 3B, 7B, and for SDSs in all chromosomes except 5A, 5B, 7A, and 7B reviewed by (Colasuonno et al., 2021).

The population developed between Endural and Aldura two cultivars with contrasting end-use quality (previously described) provides an ideal frame to detect new QTLs and to explore the effects of different prolamin alleles. Thus, the aim of this study is to analyze the rheological behaviour of the population with fast micro methods, to explore the polymorphism for prolamin alleles, and to detect genotype-phenotype associations between prolamin loci and at genome level by QTL mapping.

2.5. Investigating *Phytoene Synthase 1* and *Lipoxygenase 1* Effects on Wheat Flour Color: Clues for Breeding High-Quality Durum Wheat

Durum wheat (*Triticum turgidum* L. ssp. *Durum* 2n=28, genomes AABB) originated in the eastern Mediterranean, where it has been cultivated for the last 12000 years (Grosse-Heilmann et al., 2024). Its production represents only the 6.2% of cultivated wheat at worldwide and is mainly restricted to semiarid regions

like the Mediterranean basin (50% of world acreage production), the north American great plains, and west and central Asia (Martinez-Moreno et al., 2022). Nonetheless, durum wheat is the 10th most cultivated cereal in the world, with a global annual production ranging between 35-40 million tons (Bronccanello et al., 2023; Xynias et al. 2020). This crop differs from common wheat (*Triticum aestivum* L. subsp. *Aestivum* 2n=42, genomes AABBDD) due to its distinctive yellow grain color, higher protein content, robust gluten contends and extended shelf life. All key factors that determine durum wheat end use (Chab et al., 2024; Sissons, 2008, 2022).

Durum wheat is widely used in various products, including long and short dried pasta, fresh and sheeted pasta, couscous, bulgur and baked bread (Sissons, 2022). Pasta production is by far the most important, with around the 75% of durum wheat production allocated to that end (Grosse-Heilmann et al., 2024). Pasta quality is determined by three main factors, the raw materials, the production recipe, and the production process (Sissons, 2008; Zingale et al., 2023). The release of durum cultivars with high quality standards for pasta production has been a major breeding concern during the last half of the past century (Pagnotta et al., 2005) and is still one of the main goals of breeding programs in countries like Spain and Italy (Subira et al., 2014). Thus, traits related with durum wheat suitability for pasta production like grain protein content, gluten properties, and semolina color, have been key targets for durum wheat breeding (Zingale et al., 2023; Subira et al., 2014). Consumer preferences play an important role in directing quality traits breeding. Regarding to color, semolina and pasta with a bright yellow are preferred.

Pasta yellowness depends on the semolina carotenoid content, carotenoid degradation, and pasta processing conditions (Fu et al., 2013). The main gene controlling carotenoids biosynthesis is phytoene synthase 1 (*PSY1*), that catalyzes the key rate-limiting stage of carotenoid biosynthesis (Cazzonelly & Pogson, 2010; Ficco et al., 2014). In durum wheat other genes such as, phytoene desaturase, and zeta carotene desaturase, also play a role (Colasuonno et al., 2019). *PSY1* genes are located at 7L homoeologous chromosome arms and co-segregate with the wheat QTL explaining around the 60% of variability for carotenoids content (Singh et al., 2009). Besides, minor QTL have been detected on all durum wheat chromosomes, and several candidate genes have been associated with yellow pigment content (Colasuonno et al., 2014, 2017). Carotenoid content by itself can not accurately predict the final pasta color. Fu et al. 2011 found that elevated yellow pigment

concentration does not guarantee high yellowness of pasta, due to carotenoid degradation. This process occurs during pasta processing and is catalyzed by oxidative enzymes, among which lipoxygenases are the most characterized (Colasuonno et al., 2019). QTL analysis in durum wheat showed that between 36-54% of the variation in LPX activity is explained by the locus *LPXB-1* located on chromosome 4B (Carrera et al., 2007). This locus harbours two genes *LPXB-1.1* and *LPXB-1.2*. It has been demonstrated that the deletion of the *LPXB-1.1* copy results in LPX activity loss, producing a significant improvement in pasta color (Carrera et al., 2007). Although differences in lipoxygenase activity in semolina were not link to semolina color (Hessler et al., 2002), another *LPX* locus (*LPX-A3*), known to be expressed during grain development, has been associated with final semolina color (Carrera et al. 2007). This suggest that different LOX genes might affect different points of grain development.

Transferring this knowledge to breeding programs requires development of molecular markers, identification of superior alleles, and the estimation of allele effects and genetic interactions between genes in order to avoid setbacks. Nowadays, several molecular markers allow the identification of carotenoids biosynthetic and catalysis pathway gene alleles (MASWheat (https://maswheat.ucdavis.edu/protocols/yield_quality_traits)). But, the estimation of allele effects and interactions between different genes is still limited (Colasuonno et al., 2019). The aim of this study was, (i) to characterize for yellow pigment content a biparental population developed from two contrasting durum wheat genotypes, (ii) to determine its polymorphism for carotenoid biosynthesis and catalysis genes, and (iii) to explore the allelic effects and genetic interactions, in order to suggest the best genotypic combinations for marker assisted selection (MAS).

3. Materials and methods

3.1. Image-assisted quantification of high and low molecular weight glutenin fractions in wheat by SDS-PAGE

3.1.1. Plant material

In this study the Spanish bread wheat landraces Jeja Pardilla (JP; BGE 18202), Richela Blanca (RB; BGE 12124) and a population of 143 F_{3:5} progenies derived from the cross JP × RB were analysed. The parental lines, provided by CRF-INIA (Centro de Recursos Fitogenéticos, CSIC-INIA, Madrid), carried *Glu-A1b*, *Glu-B1e* and *Glu-D1a* alleles for the HMW-GS, differing only at the LMW-GS encoding loci. The progenies plus the two parental lines were cultivated during the season 2015-16 in plots of four rows per genotype (1 m long) at the experimental fields of the ETSIAAB, Universidad Politécnica de Madrid (40°25' N, 3°42' W, Madrid).

3.1.2. Segregating population analysis

After harvest, a grain sample of 15 g was ground in a Tekator mill for quality analysis. Bread-making quality was estimated from 1 g of whole flour by sodium dodecyl sulphate sedimentation volume test (SDSs) according to Dick & Quick (1983) with minor modifications. Technical duplicates were used in the SDSs tests. Quantification of prolamins (HMW-GS, LMW-GS and gliadins) was made by RP-HPLC following the standard protocol (Wieser et al., 1998) as described by Ribeiro et al. (2015). Briefly, the extraction was performed based on Singh et al. (1991) (described in Annex1). Then, for RP-HPLC, a RP-C8 column was used (25 cm, 4.5 mm i.d., 5 µm, Macherey-Nagel, Germany) maintained at 50 °C during the separation process and an injection volume of 50 µL for glutenins and 100 µL for gliadins was used. A gradient elution was performed, eluent A consisting of 0.1% (v/v) aqueous trifluoroacetic acid and eluent B consisting of acetonitrile and trifluoroacetic acid (99.9/0.1%, v/v), with the following elution program: 0 min 28% B, 30 min 56% B, flow rate of 1 mL/min. Detection was made by ultraviolet (UV) absorbance at 210 nm. After each analysis the column was cleaned by using 90% B for 5 minutes and equilibrated to 28% B over 10 min.

Total glutenins content was estimated by adding up HMW-GS and LMW-GS quantity. Gliadins to glutenins (GLI/GLU) and HMW-GS to LMW-GS (HMW/LMW) ratios were obtained by dividing the corresponding protein fraction quantification values.

3.1.3. LMW-GS characterization and quantification of glutenins by SDS-PAGE

Glutenin extractions were performed from 20 mg of whole flour according to Singh et al. (1991) with small modifications (Annex 1). Subsequently, HMW-GS and LMW-GS were separated by SDS-PAGE. LMW-GS alleles of parents and F_{3:5} progenies were identified by using the standard varieties from the International Wheat Consortium (Liu et al., 2010).

A subset of 22 progenies, representing the available range for RP-HPLC-derived parameters in the biparental population, plus the two parents were selected to set up a procedure to estimate HMW to LMW-GS ratio from SDS-PAGE. For these materials, glutenin extractions were performed by duplicate and two technical replicates of the SDS-PAGE by extraction were run. Richela Blanca was included as control in each gel.

Gels were imaged in a Molecular Imager® Gel Doc™ XR+ System (Bio-Rad, Hercules, California) under white light epi-illumination, optimizing the exposure time for faint bands, with ImageLab software v6.0.1 (Bio-Rad, Hercules, California). Images were saved as a scn file (Image Lab Image Document raw format) with a size of 2.8 Mb to avoid resolution loss. Gel images were analysed with ImageLab software to quantify HMW and LMW-GS. First, brightness and contrast adjustments were performed with Image transform tool to allow better visualization, these changes do not affect the raw densitometric data, nor quantification. Second, lanes and bands within each lane were automatically selected with the Lane and Bands tool. Lanes were manually curated to include the complete band lengths, and bands were manually curated adding or deleting bands when the automatic assignment was inaccurate. Third, the Lane Profile tool of ImageLab was used to determine the profile of each lane. From the profile, the volume under the peaks automatically determined by the software (peaks corresponding to detected bands) was manually curated, drawing a straight line across the baseline of the profile (Figure 8). Finally, the volume under each of the peaks, proportional to the protein amount, was obtained using the Analysis table

tool. HMW-GS quantification was determined adding up the volume of the three peaks located in the upper part of the lane. Similarly, LMW-GS amount was obtained adding up the volumes of the peaks located below (Figure 8). HMW to LMW-GS ratio was determined for each lane by dividing the obtained values. To avoid that differential staining among gels might affect further comparison between electropherograms results, all values estimated from each gel were relativized to the estimated for the Richela Blanca lane image within the corresponding gel.

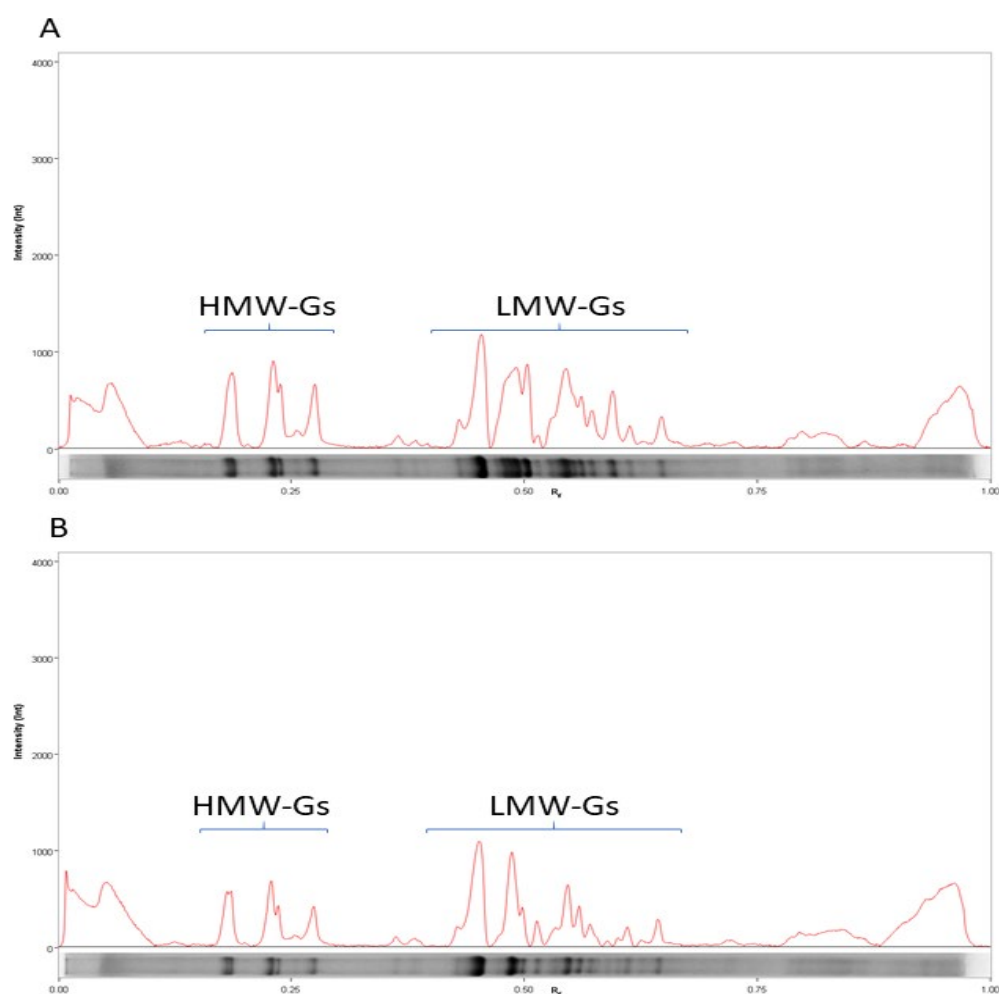


Figure 8: Quantification of glutenins by SDS-PAGE

Note: The upper part of each panel shows the densitometric analysis performed with the ImageLab software, while the corresponding lane from the SDS-PAGE is shown below. **A.** Richela Blanca. **B.** Jeja Pardilla. Both images come from the same electropherogram and are set at the same scale.

3.1.4. Statistical analysis

All statistical analyses were performed with the software R version 4.0.3 (R Core Team, 2022). Mean, standard deviation and maximum and minimum values were calculated. The Shapiro–Wilk normality test was applied to the SDSs, RP-HPLC prolamins quantification and proteins ratios (p-value = 0.01). Correlations between variables were calculated with Spearman coefficient (p-value = 0.05).

Correlations between SDS-PAGE technical replicates, prolamin extractions and SDS-PAGE with RP-HPLC were calculated with Pearson coefficient (p-value = 0.05).

3.2. Impact of quantitative and qualitative Glutenin Variation on wheat quality within a similar genetic background

3.2.1. Plant Material and glutenin characterisation

The starting plant material was the Spanish wheat (*Triticum aestivum* L.) landrace ‘Candeal’ (accession BGE026951). More than 80 grains from this accession were analysed for gliadin and glutenin composition by electrophoresis to detect biotypes (Figure 9). All the genotypes were morphologically similar, with lax and white spikes, long, rough and white awns, and white seeds (Figure 10). Individual grains carrying the same glutenin alleles were multiplied during 2019. Between two and 13 SSD (Single Seed Descent) lines were developed by biotype. SSD lines were sown in a randomised-block design with two replications during 2019-2020 season in Alcalá de Henares (Madrid) under rainfed conditions. Rainfall during the crop cycle (November to June) was 250 mm. Fertilizer was applied before sowing (150 kg.ha⁻¹ of NPK 15-15-15) and in spring (588 kg.ha⁻¹ of 26% ammonium nitrosulfate). Four varieties with varying gluten quality from different time periods were also sown as standards: ‘Marius’ and ‘Yecora’ released between 1970 and 1990, and ‘Califa Sur’ and ‘Berdun’ released after 1990.

Glutelins (glutenins) were extracted from crushed endosperm halves following a sequential procedure (Singh et al., 1991). Reduced and alkylated glutenins (HMW-GS and LMW-GS) were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 12% polyacrylamide gels. HMW-Gs allele classification was performed according to the Catalogue of Gene Symbols for Wheat (Mcintosh et al., 2013). LMW allelic variants were identified by

comparisons with test varieties proposed by Liu et al. (2010). The alleles not previously catalogued were designated as new. Gliadin electrophoresis in SDS-PAGE was also used for the identification of some LMW-GS variants based on *GLU-B3/GLI-B1* linkage following Liu et al. (2010).

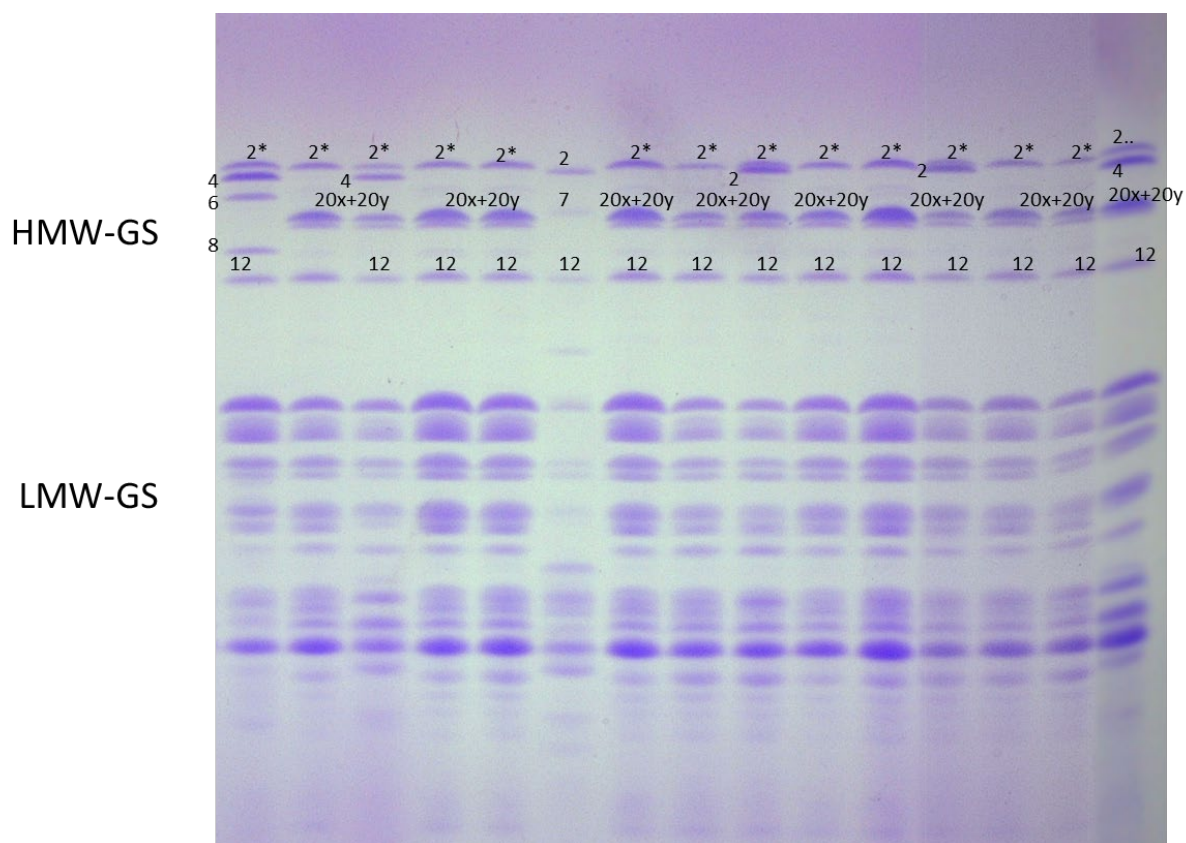


Figure 9: SDS-PAGE of different wheat grain glutenins and gliadins from the landrace Candéal (accession BGE026951)

The Figure 9 show different genotypes (gt) and the standard variety ‘Chinese Spring (CS)’, used as a reference for electrophoretic mobility. HMW-GS are identified (numbers in the gel represent glutenin subunits, designated according to the Catalogue of Gene Symbols for Wheat described in McIntosh et al., 2013).



Figure 10: Spikes of the nine genotypes (gt) analysed

3.2.2. Quality evaluation and quantification of glutenins by SDS-PAGE

Glutenin composition was verified in all the SSD lines before further analyses. Grain protein content on a dry basis (GPC, %) was determined by near-infrared reflectance analysis using an Infratec 1241 Grain Analyser (Foss Analytical, Hoganas, Sweden). Whole-grain flour samples were obtained with an LM 3100 Mill (Perten Instruments AB, Sweden). Gluten strength was evaluated on 5 g of whole grain flour samples by the sodium dodecyl sulfate sedimentation test (SDSS) using stoppered 100 ml graduated cylinders (Axford et al., 1978), samples analysed by duplicate.

HMW and LMW-GS quantification was determined by Image-assisted SDS-PAGE gels following the method described by Bouabdellah et al. (2024). Accordingly, the normalized values were obtained by using a control Protein Standard (Precision Plus Protein Standards (BIO-RAD, Madrid)) included in each gel. Thus, HMW-GS and LMW-GS content values are expressed as those proteins'

ratio with respect to the band of 75KDa from the Precision Plus Protein Standards included in all the gels. HMW to LMW-GS ratio was determined by dividing the values.

3.2.3. Statistical analyses

The effects of the glutenin alleles were analysed using a general linear mixed model (GLM) with the genotype (or *GLU*- loci) and block as fixed and random effect, respectively. The proportion of total variation explained (PVE) by the fixed effects was computed as the ratio of the effect sum of squares to the total sum of squares (%). Lines with the same glutenin genotype were considered as replicates. The calculations were conducted with the “lm4” package in R statistical software. Differences between means were tested using the t-Student test and the Satterwait’s correction if variances were not homogenous at a significance of $p = 0.05$. The effect of the glutenin fractions on the two quality parameters (SDSs and GPC) were evaluated by linear regression. The coefficient of determination (R^2), which quantifies the proportion of variation explained by the model relative to the total variation, was also calculated. The analysis was implemented with Infostat version 6.12 software (Di Rienzo et al., 2020).

3.3. A new genetic map in durum wheat, allows the detection of QTL related to yield and grain protein content

3.3.1. Population description

The plant material used in this study consisted of an $F_{4:7}$ population with 107 heterologous inbred families (HIFs) developed from a cross between the durum wheat cultivars Endural (K-63145, <http://wheatpedigree.net/>) and Aldura (K-61921, <http://wheatpedigree.net/>). Briefly, the parental lines were crossed to produce F_1 seeds that were subsequently self-pollinated. The resulting F_2 population was developed into the F_4 generation using the single seed descent (SSD) method. The derived $F_{4:7}$ population was grown during four seasons (2011–2012, 2013–2014, 2019–2020 and 2020–2021) in the experimental fields of ETSIAAB, Universidad Politécnica de Madrid (40°25’ N, 3°42’ W, Madrid). A complete randomized block design without replicates was used, with each plot consisting of four rows of 1 m in length, spaced 20 cm apart, and seeded with 3 g of seed per row.

3.3.2. Phenotypic characterization

Phenotypic characterization was carried out to evaluate a total of five grain traits and two quality-related traits in the population and parental lines. Grain size and shape data (grain area (Ar), grain perimeter (Perim), grain major ellipse (Majell) and grain minor ellipse (Minell)) were calculated as the average of the data obtained by scanning at least 300 kernels using GrainScan software (Whan et al., 2014). Thousand kernel weight (TKW) was determined by weighing 250 randomly selected grains from each plot, multiplied by four and expressed in grams. The harvested grains were milled to flour using a CT293 Cyclotec™ mill (FOSS, Denmark) equipped with a 1mm sieve. The protein content on dry matter (GPC) was estimated by near-infrared reflectance analysis (NIR) using a Perten Inframatic 9500 (Perten Instruments AB, Sweden). Whole flour color was evaluated by measuring L* (brightness, 100 = white, 0 = black), a* (+ve is redness, -ve is greenness) and b* (+ve is yellow, -ve is blue) parameters using a Minolta Chroma Meter CR-300 Chroma Meter (Biolab Sydney, Australia). The b * value, an indicator of the yellow index, was used for the analysis

3.3.3. Statistical analysis of the obtained phenotypes

Statistical analysis was performed using R v.4.0.3 (R Core Team, 2022). Mean, standard deviation, maximum, and minimum values were calculated for each trait by season. The correlations between years within each trait and correlations between traits were calculated with Spearman coefficient (p-value <0.05). Normality was tested using the Shapiro–Wilk test (p-value < 0.01), and significant traits were square root transformed to achieve normality (only Perim was transformed for QTL analysis). Homoscedasticity, or the uniformity of variances, was evaluated using the Levenne test to ensure the data suitability for further parametric analyses. ANOVA analysis was performed to identify the influence of the different seasons (p-value < 0.05). Post-hoc pairwise comparison were performed with Games-Howell test (p-value < 0.05).

3.3.4. Genotypic characterization and molecular marker selection

Genomic DNA from Endural, Aldura and the F_{4:7} population was isolated from 50 mg of whole flour according to Doyle & Doyle (1990) with small modifications. DNA was quantified in 1% agarose gels and stored at -20°C until used. High-throughput genotyping was conducted at SAGA (Servicio de Analisis

Genetico para la Agricultura, Mexico City, Mexico) using DArTseq technology (Killian et al., 2012; Sansaloni et al., 2011). Briefly, a complexity reduction method including two enzymes (PstI and HpaII) was used to create a genome representation of the set of samples. PstI-RE site specific adapter was tagged with 96 different barcodes enabling multiplexing a 96-well microtiter plate with equimolar amounts of amplification products in order to run within a single lane on Illumina HiSeq2500 instrument (Illumina Inc., San Diego, CA). The successful amplified fragments were sequenced up to 77 bases, generating approximately 500000 unique reads per sample. Thereafter the FASTQ files (full reads of 77bp) were quality filtered using a Phred quality score of 30, which represent a 90% of base call accuracy for at least 50% of the bases. More stringent filtering was also performed on barcode sequences using a Phred quality score of 10, which represent 99.9% of base call accuracy for at least 75% of the bases. A proprietary analytical pipeline developed by DArT P/L was used to generate allele calls for SNP and DArT markers (presence/absence). Then, a set of filtering were applied to select high quality markers. One of the most important parameters is the average reproducibility of markers in technical replicates for a subset of samples which was set at 99.5%. Another critical quality parameter is call rate. This is the percentage of targets that could be scored as '0' or '1', the threshold was set at 50%. After this process, the genotyping services provided two different sets of markers.

The PAV (presence/absence) markers scored as binary data (0/1) indicating the presence or absence of a marker in each accession, and the SNP (single nucleotide polymorphism) markers scored as 0/1/2 indicating the presence of the reference allele in homozygosity, the alternative allele in homozygosity, or a heterozygous genotype, respectively. To locate markers in the durum wheat reference genomes, the markers sequences were subjected to BLAST searches against the currently available *Triticum turgidum* genome Svevo v1.0 (Maccaferri et al., 2019). A marker was located according to the following criteria BLAST E-value < 5e-10 and sequence identity >90%.

From the raw data obtained, a selection of high-quality polymorphic markers was made using newly developed scripts in R. A specific selection process was applied for each marker type. For PAV markers, the first step was to discard all monomorphic markers between both parents, markers with more than 20% missing data, or markers with a minor allele frequency (MAF) below 5%. After this initial selection, the markers for which data was missing for one of the parents were identified. In such cases, an imputation was performed by assigning the allele

that was missing in the parent with known genotype to the parent with unknown genotype. A similar approach was followed for SNP markers, monomorphic markers between parents, those with more than 20% missing data or a MAF below 5% were eliminated. After this selection, the missing data for one of the parents were identified and imputed in the same way as for the PAVs. In addition, markers in which one of the parents appeared heterozygous were identified. If only homozygous for one allele, or heterozygous were present in the genotyping set, it was considered that the phenomenon was due to homeology problems between the genomes. Therefore, these markers were recoded as dominant markers (PAV) and all heterozygotes assigned the dominant allele. In rare cases, the presence of heterozygotes in one parent may be due to genotyping errors. Such cases were identified when the majority of the offspring were homozygous but polymorphic. In these cases, the heterozygous genotype of the parent was corrected by assigning the homozygous genotype that was not present in the other parent.

Once the markers were selected, the genotyping data were converted to the format required by the chosen software for genetic map construction, following the criteria listed in Table 2. PAV markers were coded as dominant, while SNPs were treated as codominant.

Table 2: Recoding of PAV and SNP markers.

SNP (codominant markers)		PAV (dominant markers)	
Code	Description	Code	Description
a	Homozygous as Endural	a	Recessive being Endural dominant
b	Homozygous as Aldura	c	Dominant equal to Endural
h	Heterozygous	b	Recessive being Aldura dominant
-	Missing data	d	Dominant equal to Aldura
		-	Missing data

3.3.5. Genetic map construction.

The genetic map was constructed using the JoinMap software version 5.0 (Van Ooijen, 2019). Marker data were filtered in JoinMap to exclude individuals or markers that showed severe segregation distortion ($p < 0.01$). The data set was reduced by including only a single representative marker per group of markers

with 100% similarity (matching genotypes across all individuals that would map to the same position). Representative loci were selected using the ‘exclude similar loci’ function in JoinMap. The recombination frequency and logarithm of odds score (LOD) were calculated for all marker pairs. Markers were grouped into linkage groups using an LOD score threshold of $3 < \text{LOD} < 12$. The resulting linkage groups were manually curated considering the physical location of markers in the durum wheat genome (*Triticum turgidum* genome Svevo v1.0 (Maccaferri et al., 2019) to accurately assign each linkage group to the corresponding durum wheat chromosome. Within each linkage group, the optimal marker sequence was determined using the regression mapping algorithm based on estimates of the recombination fraction ($r < 0.5$) and LOD scores ($\text{LOD} > 1$).

The Kosambi mapping function (Kosambi, 1944) was applied to convert the recombination fractions into genetic distances. Markers that were identified as poor fit due to creating large distances on the map were removed. As explained in the JoinMap manual, poor-fitting loci are expected to have values that are very different from the rest of the dataset. The mapping analysis was repeated until the map contained no poor-fit markers. The excluded markers were then reintroduced one by one to confirm poor fit. If reintroduction of a marker resulted in poor fit, the marker was removed from further analysis until a good quality map was established.

The constructed map was screened for collinearity for each chromosome with the physical map of the *Triticum turgidum* genome Svevo v1.0 (Maccaferri et al., 2019). This was done by visually inspect a diagram generated by plotting the genetic position of each marker on the linkage groups against the physical position on the reference genome with the software MapChart (Voorrips, 2002).

3.3.6. QTL detection.

Quantitative trait loci (QTL) detection was performed using MapQTL version 6.0 (Van Ooijen JW, 2009) integrating the genetic map with phenotypic data from the 2011–2012, 2013–2014, 2019–2020 and 2020–2021 seasons. Analysis was performed using simple interval mapping (SIM) (Lander & Botsteins, 1989), which applies the maximum likelihood method to assess the statistical association between the trait analyzed and the flanking markers. The thresholds of logarithm of odds score (LOD) for significant QTL were determined by performing 1000 permutations at the genome-wide level, obtaining a value of 2.79. Finally, as

generally applied, a stricter threshold ($LOD < 3.0$) was used to identify genomic regions significantly associated with the traits analyzed. Graphical representations of the genetic map, QTL positions, and comparative analyzes were created using MapChart software (Voorrips, 2009) to facilitate visualization and interpretation of the results.

3.3.7. Annotation of QTL regions.

Based on the physical positions of the SNP markers flanking each QTL (when available), physical intervals were defined. Genes within these intervals were obtained using the gene models for high-confidence genes reported for the durum wheat genome sequence Durum Wheat (cv. Svevo) RefSeq Release 1.0 (Maccaferri et al., 2019).

3.4. Identification of genetic factors controlling rheological properties in durum wheat by QTL detection

3.4.1. Plant material

In this study we employed 107 advanced lines from an $F_{4:7}$ population obtained by crossing the durum wheat cultivars Endural (K-63145, <http://wheatpedigree.net/>) and Aldura (K-61921, <http://wheatpedigree.net/>). The population plus the two parental lines were cultivated during the 2019-2020 and 2020-2021 seasons in the experimental fields of ETSIAAB, Universidad Politécnica de Madrid (40°25' N, 3°42' W, Madrid), using a complete randomized block design without replicates. Each block consisted of four rows 1m long and 20cm between rows, with 3g of seed per row.

3.4.2. Phenotypic characterization

Phenotypic characterization was carried out to evaluate a total of nine traits related with flour end-use quality. The characterized traits included; quantification of HMW-GS, LMW-GS and estimation of HMW-GS to LMW-GS ratio; gluten strength estimation; and percentage of solvent retention capacity for five different solvents.

The harvested grains were milled to flour using a CT293 Cyclotec™ mill (FOSS, Denmark) equipped with a 1mm sieve. From whole flour, gluten strength

was determined by duplicate on 1g of whole grain flour samples by a sodium dodecyl sulfate-sedimentation volume test, according to Dick & Quick (1983) with minor modifications.

HMW and LMW-GS quantification was determined by Image-assisted SDS-PAGE gels from 20mg of whole flour following the method described by Bouabdellah et al. (2024) (described previously in this thesis, Annex 1). Accordingly, the normalized values were obtained by dividing by the same Endural sample included in each gel. Thus, HMW-GS and LMW-GS content values are expressed as those proteins' ratio with respect to Endural included in all the gels. HMW to LMW-GS ratio was determined by dividing their values.

The SRC (solvent retention capacity) tests were performed by duplicate for each sample following the modified protocols of the AACC Methods 56-10 and 56-11 described by Guzman et al. (2015). That analyzed water SRC (distilled water, from now on named S1), alkaline water SRC (Sodium bicarbonate (NaHCO_3) solution (0.1N), from now on S2), lactic acid SRC (lactic acid solution (5%), from now on S3), sodium carbonate SRC (sodium carbonate solution (5%), from now on S4) and sucrose SRC (sucrose solution (50%), from now on S5). First, a 2.0 ml empty centrifuge tube was weighed. Second 0.3g of whole flour of known moisture content was added to the tube (exact weigh also determined) and mixed with 1.5 ml of the respective solvent. Third, tubes were immediately put in a Thermomixer at 1400 rpm for 5 minutes, at 25°C. Then the samples were centrifuged at $4000 \times g$ for 2 minutes and the supernatant was carefully discarded. Fourth, the tubes were allowed to drain for 10 minutes at room temperature and the lids were dried with cloth paper prior to weighing. Finally, SRC values were calculated as a percentage using the following equation:

$$\% \text{SRC} = \left(\frac{\text{Tube and gel weight} - \text{empty tube weight}}{\text{Flour weight}} \right) \left(\frac{86}{100 - \text{Flour moisture}} \right) - 1 \times 100$$

3.4.3. Prolamin genotyping

Endural and Aldura prolamin alleles were previously known (Table 3). For the F_{4:7} population polymorphic glutenins and gliadins genotypes were determined for each accession using the parents as reference.

Table 3: Detail of the glutenins and gliadins alleles and subunits as previously analyzed by Dr. Marta Rodriguez-Quijano (personal communication). Polymorphic loci indicated in bold include the different protein subunits.

Loci	Endural	Aldura
<i>GLU-A1</i>	<i>c (null)</i>	<i>c(null)</i>
<i>GLU-B1</i>	<i>e</i> (20x+20y)	<i>d</i> (6+8)
<i>GLU-A3</i>	<i>l</i> (6+10)	<i>k</i> (5)
<i>GLU-B3</i>	<i>w</i> (2+4+15+17)	<i>s</i> (8+9+13+16)
<i>GLU-B2</i>	<i>a</i> (12)	<i>b</i> (null)
<i>GLI-A1</i>	<i>c</i>	<i>c</i>
<i>GLI-B1</i>	<i>c</i> (ω-35/γ-45)	<i>a</i> (ω-33,35,38/γ-42)
<i>GLI-A2</i>	α(α-block+α-10)	<i>a</i>(α-block+α-11)
<i>GLI-B2</i>	γ-1+δ-4	γ-2+δ-3+δ-5
<i>GLI-B3</i>	<i>null</i>	<i>null</i>

Glutenins were extracted following a sequential procedure (Singh et al., 1991). Reduced and alkylated glutenins (HMW-GS and LMW-GS) were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 12% polyacrylamide gels (detailed protocol described in Annex 1). *GLU-B1*, *GLU-A3*, *GLU-B3*, *GLU-B2* allelic variants were identified by comparisons with the parent cultivars.

Gliadins were extracted from single seeds and fractionated in acid (pH 3.1) polyacrylamide gel electrophoresis (A-PAGE) according to Lafandra and Kasarda (1985). *GLI-B1*, *GLI-A2* and *GLI-B2* allelic variants were identified by comparisons with the parent cultivars.

3.4.4. Statistical analysis

Statistical analysis was performed using R v.4.0.3 (R Core Team, 2022). Mean, standard deviation, maximum, and minimum values were calculated for each trait by season. Differences between seasons were detected by the Mann-Whitney test (p -value < 0.05). The correlations between years within each trait, and correlations between traits, were calculated with Spearman coefficient (p -value < 0.05). Normality was tested using the Shapiro–Wilk test (p -value < 0.01), only S3, S5 and LMW-Gs quantity were normally distributed. Significant traits were inverse transformed to achieve normality if possible. S1, S2 and HMW-GS quantity were transformed for QTL analysis. S4, HMW-GS to LMW-GS ratio and SDSs did not achieve normality by transformation, thus nonparametric statistics were used.

To determine if there were associations between the different traits with glutenins and gliadins loci, Wilcox test (p -value < 0.05) was used, analyzing the data from each season separately.

3.4.5. Detection and annotation.

Quantitative trait loci (QTL) detection was performed using MapQTL version 6.0 (Van Ooijen JW, 2009) integrating, the PAV and SNP genotypes for each F_{4:7} (data only available for 105 F_{4:7}) and the genetic map (previously described), with the nine traits data from the 2019–2020 and 2020–2021 seasons. For all the traits normally distributed, or that achieved normality by inverse transformation, the analysis was performed using simple interval mapping (SIM) (Lander & Botsteins, 1989), with a threshold of logarithm of odds score (LOD > 3.0). For traits that did not follow a normal distribution (S4, HMW-GS to LMW-GS ratio and SDSs), the analysis was performed using Kruskal-Wallis test procedure from MapQTL. The K threshold to consider marker associated with the trait varied depending on the degrees of freedom (Df). For PAV (Df = 1) or SNPs (Df = 2) threshold was set to 8 or 11 respectively.

Graphical representations of the genetic map and QTL positions were created using MapChart software (Voorrips, 2009). Annotation features of the QTL genomic regions were based on the physical positions of the markers flanking each QTL (when available). Only high-confidence genes reported for the durum wheat genome sequence durum Wheat (cv. Svevo) RefSeq Release 1.0 (Maccaferri et al., 2019) were considered.

3.5. Investigating *Phytoene Synthase 1* and *Lipoxygenase 1* Effects on Wheat Flour Color: Clues for Breeding High-Quality Durum Wheat

3.5.1. Plant Material

In this study the durum wheat cultivars Endural (K-63145, <http://wheatpedigree.net/>), Aldura (K-61921, <http://wheatpedigree.net/>) and a F_{4:7} population of 104 advanced lines derived from the cross Endural × Aldura (single seed descent method) were analysed. The population plus the two parental lines were cultivated during the seasons 2019-2020 and 2020-2021 at the experimental fields of the ETSIAAB, Universidad Politécnica de Madrid (40°25' N, 3°42' W, Madrid), using a randomized complete block design without replicates. Each block consisted of four rows 1m long and 20cm between rows, with 3g of seed per row.

3.5.2. Phenotypic characterization

After harvest a sample of 15 g was ground in a Tekator mill equipped with a 1 mm sieve. Whole flour color was evaluated by measuring L* (brightness, 100 = white, 0 = black), a* (+ve is redness, -ve is greenness), and b* (+ve is yellow, -ve is blue) parameters by means of a Minolta Chroma Meter CR-300 Chroma Meter (Biolab Sydney, Australia).

Whole grain flour total yellow pigment content (YPC) and pigment loss in dough (after exposure to oxygen and light) were estimated with the micro-scale method based on the AACC approved method 14-50 adapted by Fu et al. (2013). For YPC, 1 ml of water saturated n-butanol was added to each 0.2 g sample, the mixture was homogenised (5 min), followed by resting in darkness (1 h), vortexing (15 s), and centrifugation (at 15000g for 10 min). Absorbance of the supernatant obtained was measured individually for two aliquots from each sample extract at 436 nm using a SPECTROstar® nano (BMG Labtech Ortenberg, Germany), values were averaged and converted to yellow pigment using the coefficient for β -carotene prior to data analysis. To estimate total yellow pigment content in dough (YPCd) and yellow pigment loss in dough (YPL %), 0,15 ml of water was added to 0,2 gr of whole flour and homogenized (5 min), the resulting dough was rested for 2 h with an open cover, with continuous flow of air and at room temperature to allow the oxidation of pigment dough. Later, YPCd was determined by adding 0.85 ml of pure

1-butanol, followed by the protocol described above for YPC. Both parameters were determined in duplicate for all samples. YPL % was the difference in pigment content between YPC and YPCd expressed as a percentage of this difference in YPC.

3.5.3. Identification of polymorphic genes and population genotyping

Genomic DNA from Endural, Aldura and the population was isolated from 50 mg of whole flour according to Doyle & Doyle (1990) with small modifications. The DNA quality was assessed in 1% agarose gels and stored at -20°C until used.

Accessions Endural and Aldura were screened for polymorphism on key genes related with carotenoids biosynthetic pathway and carotenoid catalysis reviewed by (Colasuonno et al., 2019). Phytoene synthase 1 genes (*PSY1-A1* and *PSY1-B1*) were genotyped with the *Psy1-A1*_STS marker (Singh et al. 2009) that differentiates alleles *Psy1-A1a*, *Psy1-A1l* and *Psy1-A1o*, as well as, *Psy1-B1n* and *Psy1-B1o*. Briefly, both genes were amplified by PCR (fw GTGGATATTCCCTGTCAGCATC, rev GCCTCCTCGAAGAACATCCTC) and products were separated on an 8% polyacrylamide gel. Phytoene desaturase B (*PDSB*) and zeta carotene desaturase A (*ZDSA*) were genotyped as described by Rasheed et al., (2016) and Pasten et al., (2021) respectively. Lipxygenase genes (*LPX-B1.1* and *LPX-A3*) were genotyped as described by Carrera et al. (2007). Briefly, *LPX-B1.1* was amplified with primers LOXA-L/R (fw CTGATCGACGTCAACAAC, rev CAGGTACTCGCTCACGTA) that produced two fragments around 800pb when the allele not carrying a deletion in the *LPX-B1.1* gene was present (*LPX-B1.1a*), and only a band in homozygote individuals for the allele carrying the deletion (*LPX-B1.1c*). *LPX-A3* gene was amplified with primers LOXB-L/R (fw CACGATAACTTCATGCCAT, rev ACTCCTCCAGCTCCTTGT) that produced two fragments of 930 and 920pb. The 920pb fragment was later digested with HaeII restriction enzyme, that discriminates an SNP inside *LPX-A3* (920pb) gene. PCR products or digestions were separated on a 1% agarose gel.

All PCRs reactions were carried out with the DNA AmpliTools Complex Master Mix (Biotools, Madrid) following the manufacturer's protocol. Molecular markers polymorphic between Endural and Aldura were screened in the segregating population as described above.

3.5.4. Statistical analysis

All statistical analyses were performed with the software R version 4.0.3 (R Core Team, 2022). Mean, standard deviation, maximum and minimum values were calculated. The Shapiro–Wilk normality test was applied to the different traits (p-value < 0.01). Variance homeostocasticity was tested with Levenne test (p-value < 0.01). Correlations between seasons for each trait and between traits were calculated with Spearman coefficient (p-value < 0.05). ANOVA analysis were performed to identify the influence of the different genes, environments and the interactions among genes or genotype by environment (p-value < 0.05). When ANOVA was significant, a Duncan's test (P-value < 0.05) and a Tukey HSD post hoc test (p-value < 0.05) were subsequently performed to compare within the same year. The effects of the different alleles for each gene or allele combinations were estimated by Linear Mixed-Effects Model (LME), setting the year as random effect, and the genotype as fixed effect

4. Results

4.1. Image-assisted quantification of high and low molecular weight glutenin fractions in wheat by SDS-PAGE

4.1.1. Quality and genotypic characterization of Richela Blanca × Jeja Pardilla population

In order to properly set up any new protocol to evaluate a phenotypical trait is necessary to test it in a collection of samples presenting a wide range of variation, that will guarantee that the developed methodology can be extensively used. Thus, in the present work we have first characterized our experimental population plus the parental lines at genetic and phenotypical level.

SDS-PAGE was used to characterize the glutenin alleles and confirmed that for HMW-GS both lines carried the *Glu-A1b*, *Glu-B1e* and *Glu-D1a* alleles. Regarding LMW-Gs, parents were monomorphic for *GLU-B3* locus, both carrying allele i, and polymorphic for *GLU-A3* and *GLU-D3*. Richela Blanca presented the *Glu-A3d* and *Glu-D3b* alleles, while Jeja Pardilla presented null alleles for both loci (*Glu-A3f* and *Glu-D3c*). F_{3:5} lines presented polymorphism at both loci (Table 4); it must be noted that, as Jeja Pardilla carried null alleles at both loci, heterozygotes cannot be distinguished.

Table 4: LMW-GS alleles segregation in the F₃:F₅ progenies and the selection subset (including 22 progenies plus the parents).

<i>GLU-A3</i>	<i>GLU-D3</i>	F _{3:5} progenies	Selection
<i>dd, df</i>	<i>bb, bc</i>	60	10
<i>dd, df</i>	<i>cc</i>	47	6
<i>ff</i>	<i>bb, bc</i>	31	7
<i>ff</i>	<i>cc</i>	5	1

In regard to quantitative analyses, we determined several parameters related with protein content and rheological properties of the flour in samples obtained during season 2015/2016. Richela Blanca and Jeja Pardilla presented clear differences at SDSs test (for which they differed around 40 mm), and gliadins, total glutenins and LMW-GS contents, as estimated by RP-HPLC, but showed

similar contents of the non-polymorphic HMW-GS. The derived population presented transgressive values for all the traits analyzed and a wide range of variation (Table 5). We then explored the correlation between the different variables analyzed (Figure 11). SDSs values were weakly correlated with LMW-GS and total glutenins, but not correlated with HMW-GS. Besides, protein fractions ratios clearly reflected the different protein amounts.

Table 5: Means and ranges of the parameters determined by SDSs test and RP-HPLC in Jeja Pardilla, Richela Blanca, their derived population (Pop) and the genotypes selected to set up the SDS-PAGE methodology (including Jeja Pardilla, Richela Blanca and 22 F_{3:5} progenies from the population). SDSS: sodium dodecyl sulphate sedimentation volume test, GLI: gliadins quantification, HMW: high molecular weight glutenins quantification, LMW: low molecular weight glutenins quantification, GLU: total glutenins quantification, GLI/GLU: gliadins to glutenins ratio. HMW/LMW: high molecular weight to low molecular weight glutenins ratio.

	SDSS	GLI (g/100g)	HMW (g/100g)	LMW (g/100g)	GLU (g/100g)	Ratio GLI/GLU	Ratio HMW/LMW
Richela Blanca	82.50	11.10	0.60	2.88	3.50	3.19	0.21
Jeja Pardilla	42.00	14.80	0.68	1.98	2.70	5.55	0.34
Pop-Average	42.29±1.02	12.87±0.24	0.62±0.01	1.92±0.03	2.53±0.04	5.34±0.15	0.33±0.01
Pop-Range	20.00-83.00	4.90-18.90	0.32-1.09	1.00-3.05	1.40-3.90	1.98-11.43	0.18-0.54
Selection Average	44.29±2.63	13.17±0.59	0.53±0.03	1.84±0.1	2.37±0.11	6.07±0.48	0.30±0.02
Selection Range	26.50-82.50	6.40-18.10	0.32-0.81	1.00-2.88	1.40-3.50	1.98-11.43	0.18-0.50

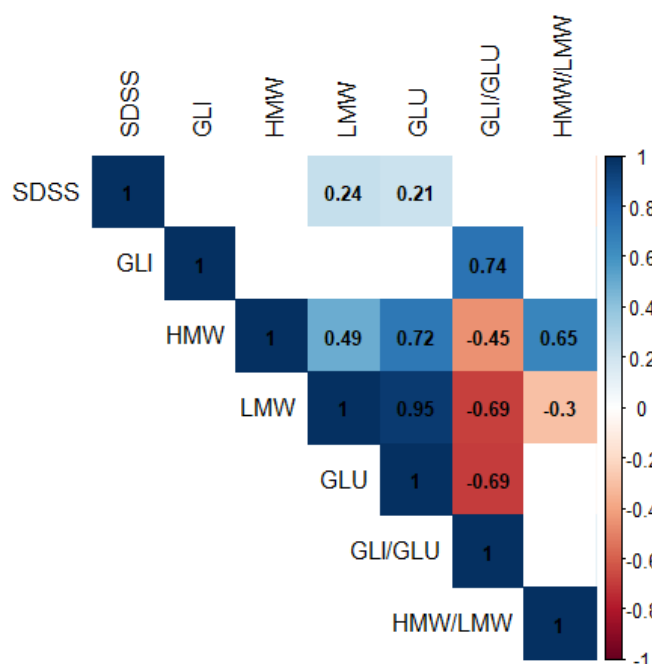


Figure 11: Correlation analyses among different traits

Note: Only significant values are shown. SDS: sodium dodecyl sulphate sedimentation volume test and parameters estimated by RP-HPLC, GLI: gliadins quantification, HMW: high molecular weight glutenins quantification, LMW: low molecular weight glutenins quantification, GLU: total glutenins quantification, GLI/GLU: gliadins to glutenins ratio. HMW/LMW: high molecular weight to low molecular weight glutenins ratio.

4.1.2. Setting up a new methodology for glutenins quantification from SDS-PAGE

In order to test the repeatability of our quantification methodology, we compared prolamin content estimations derived from electropherogram replicates. It resulted in correlation values of 0.84, 0.9 and 0.87 for HMW-GS, LMW-GS and total glutenins, respectively (p-values 3.035×10^{-13} , 2.2×10^{-16} , 5.229×10^{-15}). Then, we obtained the average between SDS-PAGE-based estimates and further compared the prolamins extraction duplicates. In this case correlations were still significantly positive (0.7, 0.9 and 0.84, for HMW-GS, LMW-GS and total glutenins, p-values 0.0002324, 4.622×10^{-9} , 3.554×10^{-7}), but a little lower than between gel replicates for HMW-GS. Our results demonstrate that our procedure for image-derived prolamins quantification is highly repeatable. It is also shown by the coefficients of variation among the total four replicates by flour sample, that were lower than 10% except for one HMW-GS sample.

Once the repeatability was established, we compared our methodology with the standard RP-HPLC protocol. It must be noted that while the same flour samples were used for SDS-PAGE and RP-HPLC, different protein extractions were performed. Correlations were significantly positive for HMW-GS and LMW-GS (0.45 and 0.43 respectively, p-values 0.03168, 0.04045), but not significant for total glutenins ($r=0.21$ p-value=0.34). This lower correlation for total glutenins

might be caused by the accumulation of HMW-GS and LMW-GS deviations, especially considering that there is not polymorphism for HMW-GS.

Finally, we explored if the associations between SDSs test values and glutenins amounts previously detected by RP-HPLC were maintained with the SDS-PAGE-based quantification values. As by RP-HPLC, HMW-GS were not correlated with SDSs, while LMW-GS and total glutenins showed a significant positive correlation with SDSS (0.48 and 0.47 respectively, p -values= 0.01936, 0.02416) that was even higher than previously detected by RP-HPLC.

4.1.3. HMW/LMW ratios estimated by RP-HPLC and SDS-PAGE

We calculated the HMW to LMW-GS ratio directly from gel image estimates. In this case, no standardization by dividing for Richela Blanca values was performed, as any differences due to SDS-PAGE staining or extraction should equally affect HMW and LMW-GS, being compensated when the ratio among them is calculated. Correlations for this ratio among SDS-PAGE duplicates, on one side, and glutenins extractions duplicates, on the other, were highly positive and significant (0.98 and 0.97 respectively, p -values < $2.2e-16$, = $3.156e-14$) (Figure 12A, B). Moreover, coefficients of variation among the total four replicates by flour sample were lower than 4% except for one case. Finally, when we compared with the values estimated by RP-HPLC, we observed a very highly significant correlation of 0.86 (p -value $7.449e-08$) (Figure 12C).

To finish, we analyzed the association between SDSs volume values and the HMW/LMW-GS ratio estimated by SDS-PAGE, obtaining a significant negative correlation ($r=-0.56$, p -value=0.004). The reasons underlying this result are discussed below.

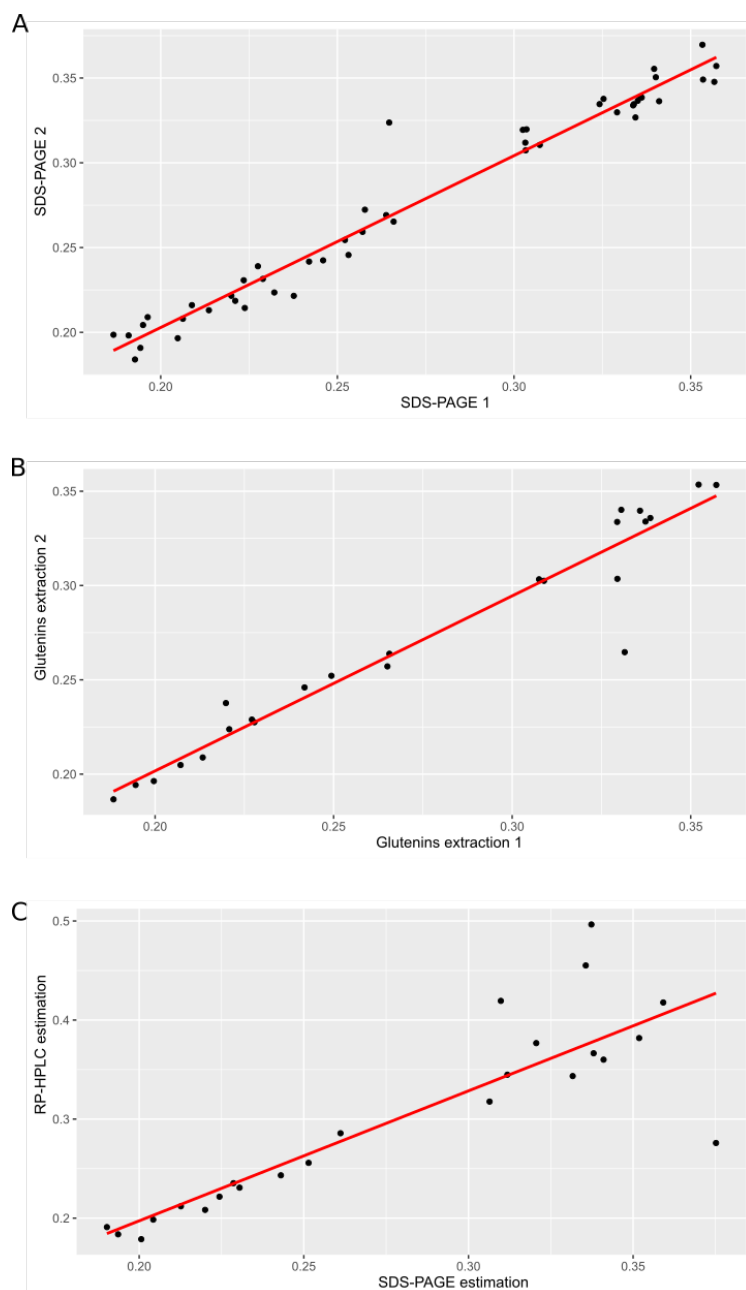


Figure 12: Correlation analyses between HMW/LMW ratio estimates

Note: **A.** Correlation between SDS-PAGE duplicates. **B.** Correlation between glutenins extraction duplicates. **C.** Correlation between SDS-PAGE and RP-HPLC results.

4.2. Impact of quantitative and qualitative Glutenin Variation on wheat quality within a similar genetic background

4.2.1. Glutenin characterisation

Analysis of prolamins (gliadins) and glutelins (glutenins) in the wheat landrace ‘Candeal’ (accession BGE026951) revealed the presence of nine biotypes (genotypes) primarily differing in their HMW-GS (Figure 9). Allelic variation at the *GLU-1* and *GLU-3* loci, coding for HMW-GS and LMW-GS, respectively was identified by electrophoresis using reference varieties and linkage to ω -gliadins encoded at *GLI-B1*, following Liu et al. (2010) (Table 6, Figure13, 14 and 15). Some allelic variants (designated as new) did not correspond to any previously described alleles. The genotypes (gt) identified as gt 1, gt 2, and gt 3, which differed only in their *GLU-D1* alleles, had the highest frequency (combined frequency of 65%), whereas gt 7, gt 8 and gt 9 were rare (frequency < 5%). Among the nine genotypes analysed, the most frequent *GLU-1* alleles were *Glu-A1b* (89%), *Glu-B1e* (94%) and *Glu-D1c* (43%). Three genotypes (gt 3, gt 7, and gt 9) had these three most frequent alleles. At *GLU-3* loci, the most common alleles were *Glu-A3new2* (70%), *Glu-B3new1* (70%) and *Glu-D3c* (72%).

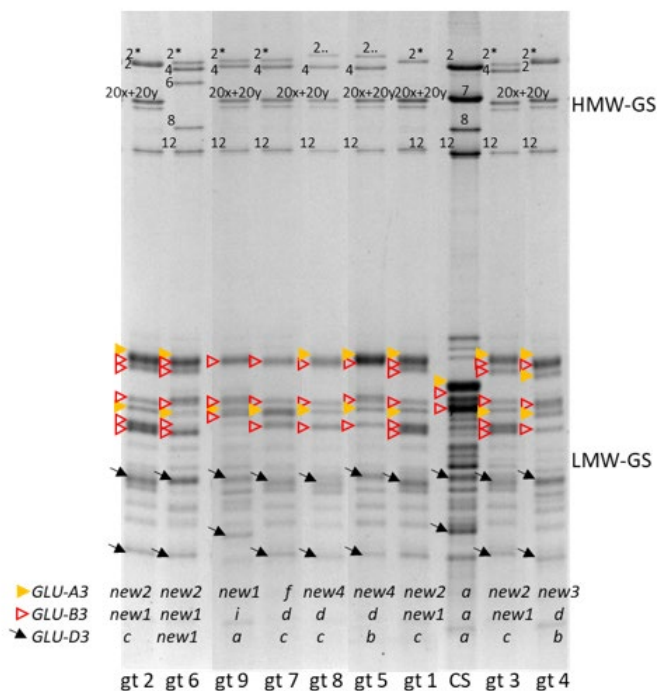


Figure 13: SDS-PAGE of HMW and LMW-GS of the genotypes analysed (gt 1-gt 9)

Note: HMW-GS subunits and the allelic variants of LMW-GS at the *GLU-3* loci are indicated. Those allelic variants not identified previously were designated as *new*. CS indicated the standard variety ‘Chinese Spring’.

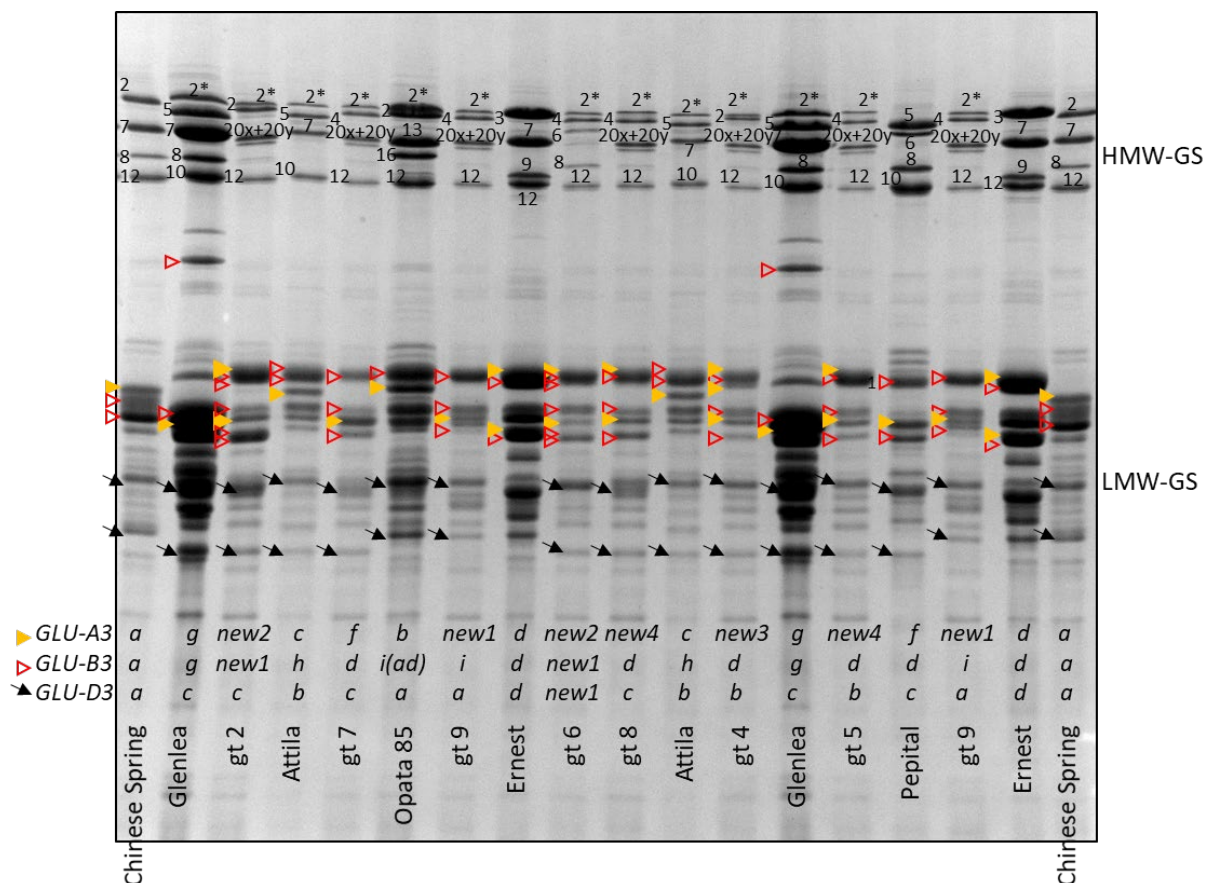


Figure 14: SDS-PAGE of glutenins of some genotypes analysed

Note: genotypes analysed (gt 2, gt 4, gt 5, gt 6, gt 7, gt 8, and gt 9) and the standard wheat varieties (CS: Chinese Spring, Glenlea, Attila, Opata 85, Ernest, and Pepital), used as references for electrophoretic mobility. The HMW-GS and the allelic variants at the *GLU-3* loci are indicated. The allelic variants of the standard varieties were identified by Liu et al. (2010). Novel allelic variants were designated as *new*.

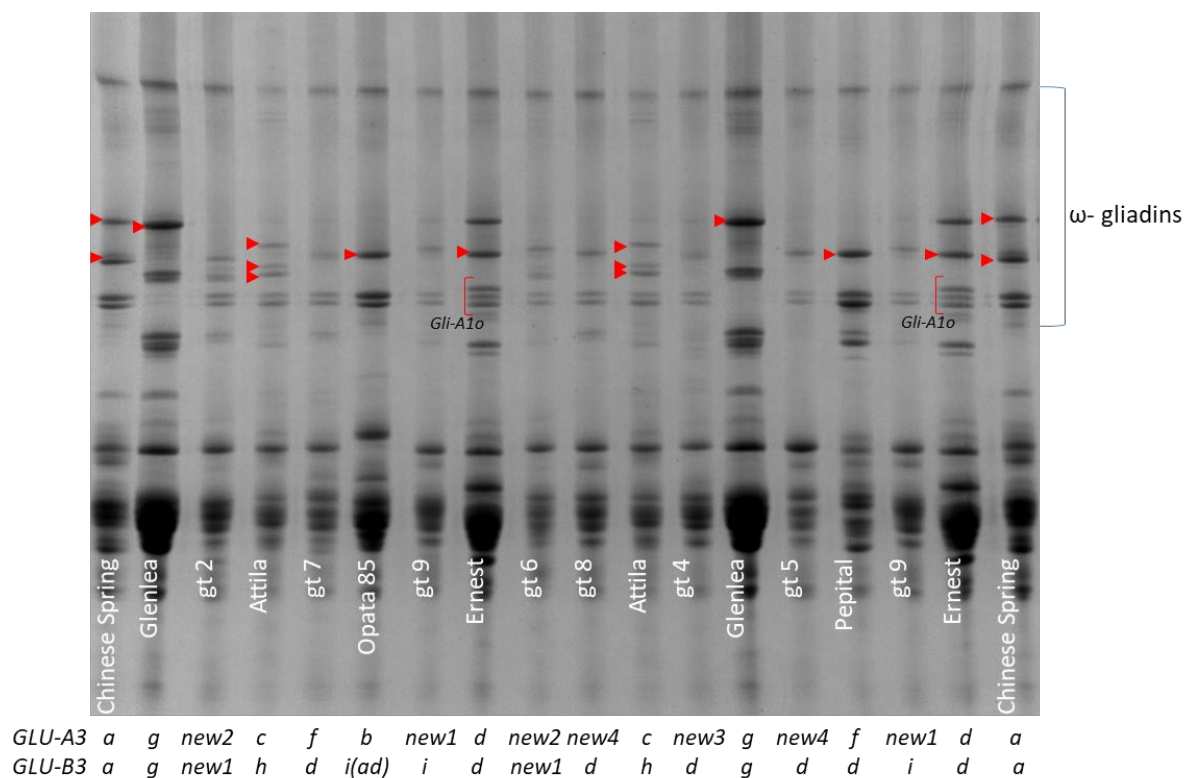


Figure 15: SDS-PAGE of gliadins of the same genotypes

Note: (gt) and standard varieties shown in Figure. 14. The arrows (▶) indicate the bands corresponding to the *GLI-B1* alleles. The ω - gliadins ([]) and the allele *Gli-A1o* ([]) are also identified. The allelic variation at *GLU-A3* and *GLU-B3* for each sample is indicated at the bottom, below the gel image.

Table 6: Genotype (gt) frequency and allele variation at the *GLU* loci of the genotypes analysed. Alleles were designated with letters according to the Catalogue of Gene Symbols for Wheat. Alleles not previously described were named as *new*.

gt	No. lines	gt, %	HMW-GS Allele (subunits)			LMW-GS Allele		
			<i>GLU-A1</i>	<i>GLU-B1</i>	<i>GLU-D1</i>	<i>GLU-A3</i>	<i>GLU-B3</i>	<i>GLU-D3</i>
1	13	24.53	<i>b</i> (2*)	<i>e</i> (20x+20y)	<i>l</i> (12)	<i>new2</i>	<i>new1</i>	<i>c</i>
2	11	20.75	<i>b</i> (2*)	<i>e</i> (20x+20y)	<i>a</i> (2+12)	<i>new2</i>	<i>new1</i>	<i>c</i>
3	10	18.87	<i>b</i> (2*)	<i>e</i> (20x+20y)	<i>c</i> (4+12)	<i>new2</i>	<i>new1</i>	<i>c</i>
4	6	11.32	<i>b</i> (2*)	<i>e</i> (20x+20y)	<i>a</i> (2+12)	<i>new3</i>	<i>d</i>	<i>b</i>
5	4	7.55	<i>y</i> (2..)	<i>e</i> (20x+20y)	<i>c</i> (4+12)	<i>new4</i>	<i>d</i>	<i>b</i>
6	3	5.66	<i>b</i> (2*)	<i>d</i> (6+8)	<i>c</i> (4+12)	<i>new2</i>	<i>new1</i>	<i>new1</i>
7	2	3.77	<i>b</i> (2*)	<i>e</i> (20x+20y)	<i>c</i> (4+12)	<i>f</i>	<i>d</i>	<i>c</i>
8	2	3.77	<i>y</i> (2..)	<i>e</i> (20x+20y)	<i>c</i> (4+12)	<i>new4</i>	<i>d</i>	<i>c</i>
9	2	3.77	<i>b</i> (2*)	<i>e</i> (20x+20y)	<i>c</i> (4+12)	<i>new1</i>	<i>i</i>	<i>a</i>
Berdun	1	-	<i>a</i> (1)	<i>i</i> (17+18)	<i>a</i> (2+12)	<i>c</i>	<i>c</i>	<i>c</i>
Califa Sur	1	-	<i>a</i> (1)	<i>al</i> (7oe+8*)	<i>d</i> (5+10)	NA	NA	NA
Marius	1	-	<i>c</i> (Null)	- (7*+9)	<i>c</i> (4+12)	<i>d</i>	<i>f</i>	<i>c</i>
Yecora	1	-	<i>a</i> (1)	<i>i</i> (17+18)	<i>d</i> (5+10)	<i>c</i>	<i>b</i>	<i>b</i>

4.2.2. Relationship between glutenin composition, quality traits and glutenin fractions

4.2.2.1. Effects of HMW and LMW genotype on quality traits

Mean values for SDSs among the genotypes ranged from 14.88 mm (gt 7) to 31.68 mm (gt 2), and for GPC, from 8.47 % (gt 4) to 10.71% (gt 1) (Table 2). In the four standard varieties, SDSS values ranged from 34.50 mm ('Marius') to 55 mm ('Yecora'), and GPC values ranged from 9.75 % ('Marius') to 11.9% ('Yecora') (Table 7).

Genotype had a highly significant effect and explained the main part of the variation for SDSs and GPC (Table 7). The *GLU-D1* and *GLU-A3* loci had a significant effect on both quality traits, with the *GLU-D1* locus explaining most of the variation (about 80%). Mean comparisons among genotypes differing only at the *GLU-D1* locus (gt 1, gt 2, and gt 3) enable ranking of alleles for performance: *a* (gt 2) > *l* (gt 1) > *c* (gt 3) for SDSs, and *l* (gt 1) > *a* (gt 2) > *c* (gt 3) for GPC (Table 7). Mean comparisons of genotypes with the same *GLU-D1* allele but differing at the *GLU-3* loci (i.e. gt 2 and gt 4 for *Glu-D1a*; gt 3, gt 7, and gt 9 for *Glu-D1c*) showed

deviations from expected performance. For instance, gt 9 exhibited unexpectedly higher SDSs values, while gt 4 displayed lower SDSs and GPC values relative to gt 2. Comparisons between gt 5 and gt 8, differing only in the *GLU-D3* alleles (*b* and *c*), revealed no significant differences.

Table 7: Mean and standard error of the quality tests and glutenin fractions for the genotypes (gt) analysed. Glutenin allelic variation at *GLU-1* (*GLU-A1*, *GLU-B1*, *GLU-D1*) and *GLU-3* (*GLU-A3*, *GLU-B3*, *GLU-D3*) is indicated.

gt	<i>GLU-1/GLU-3</i> alleles	SDSS (ml)	GPC (%)	HMW/LMW ratio ^a	HMW-GS	LMW-GS
1	<i>b,e,l/new2,new1,c</i>	19.46±0.23c	10.71±0.22a	0.215±0.004i	2.59±0.04i	12.14±0.36ab
2	<i>b,e,a/new2,new1,c</i>	31.68±0.43a	9.75±0.13b	0.253±0.00bcdef	3.22±0.07b	12.55±0.28ab
3	<i>b,e,c/new2,new1,c</i>	15.63±0.41ef	8.71±0.04c	0.238±0.010gh	3.01±0.07efh	12.71±0.35ab
4	<i>b,e,a/new3,d,b</i>	18.46±0.38d	8.47±0.1e	0.249±0.002fg	3.01±0.02cgh	12.10±0.09b
5	<i>y,e,c/new4,d,b</i>	15.44±0.73ef	9.23±0.15d	0.267±0.004ae	3.15±0.06bde	11.79±0.36b
6	<i>b,d,c/new2,new1,new1</i>	15.75±0.25f	9.23±0.34bcd	0.280±0.010a	3.33±0.12abc	11.89±0.12b
7	<i>b,e,c/f,d,c</i>	14.88±0.38f	9.48±0.43abcd	0.269±0.010ac	3.30±0.1abf	12.24±0.59ab
8	<i>y,e,c/new4,d,c</i>	16.88±0.13de	9.23±0.08bd	0.269±0.010adf	3.31±0.09abdf	12.32±0.29ab
9	<i>b,e,c/new1,i,a</i>	24.00±0.25b	10.23±0.33abc	0.270±0.010ab	3.64±0.14ag	13.50±0.14a
Berdun	<i>a,i,a/c,c,c</i>	49.50±0.50	10.45±0.05	0.324±0.00	4.07±0.00	12.57±0.01
Califa Sur	<i>a,al,d/NA</i>	43.00±0.00	NA	0.425±0.00	5.35±0.03	12.60±0.05
Marius	<i>c,7*+9,c/d,f,c</i>	34.50±0.50	9.75±0.05	0.272±0.00	3.88±0.01	14.24±0.03
Yecora	<i>a,i,d/NA</i>	55.00±0.00	11.9±0.00	0.264±0.00	4.19±0.02	15.86±0.10

Means followed by different letters are significantly different at $P < 0.05$.

SDSS - SDS Sedimentation Test, GPC – Grain Protein Content, HMW - High Molecular Weight, LMW - Low Molecular Weight, GS - Glutenin Subunit, NA - not available a HMW -GS and LMW - GS values are expressed as those proteins ratio with respect to the band of 75KDa from the Precision Plus Protein Standards (BIORAD) included in all the gels.

Table 8: Variation (PVE %) of the quality parameters (SDSs and GPC) and protein fractions explained by the genotype and the glutenin loci.

Variable	SDSS	GPC	HMW/LMW ratio	HMW-GS	LMW-GS
genotype	91	55	70	51	ns
<i>GLU-A1</i>	ns	ns	ns	ns	ns
<i>GLU-B1</i>	ns	ns	23	7	ns
<i>GLU-D1</i>	84	77	61	73	ns
<i>GLU-A3</i>	15	21	16	20	ns
<i>GLU-B3</i>	ns	ns	ns	ns	ns
<i>GLU-D3</i>	ns	ns	ns	ns	ns

SDSS - SDS Sedimentation Test, GPC – Grain Protein Content, HMW - High Molecular Weight, LMW - Low Molecular Weight, GS - Glutenin Subunit. ns - Non-significant

The individual effects of *GLU-D1* and *GLU-D3* alleles were also examined by comparing the mean values across genotypes carrying each allele (Table 9). Results confirmed the ranking of *GLU-D1* alleles for SDSs as $a > l > c$ ($p < 0.001$), and demonstrated the superiority of *Glu-D1l* over *Glu-D1a* and *c* for GPC ($p < 0.001$). For *GLU-D3* alleles, *c* displayed higher SDSs and GPC values than *b*. The limited number of lines for some allelic combinations precluded valid comparisons for other alleles.

4.2.2.2. Effects of HMW and LMW genotype on HMW /LMW ratio

Mean HMW/LMW ratio values varied from 0.215 (gt 1) to 0.280 (gt 6) among biotypes (Table 7) and from 0.264 ('Yecora') to 0.425 ('Marius') among varieties (Table 7).

Genotype accounted for 70% of HMW/LMW ratio variability (Table 8), with significant contribution from *GLU-D1*, *GLU-A3* and *GLU-B1* loci displayed a significant effect. The *GLU-D1* explained the largest proportion of the total variability, followed by *GLU-B1* and *GLU-A3*.

When comparing genotypes differing only at *GLU-D1* locus (gt 1, gt 2, and gt 3), the rank order of alleles was $a > c > l$ (Table 7). For genotypes carrying the same *GLU-D1* allele but differing at the *GLU-3* loci, gt 4 exhibited a value similar to gt 2 (both with *Glu-D1a*), whereas gt 3 had the lowest value among genotypes with *Glu-D1c* (gt 3, gt 9, and gt 7) (Table 7).

When averaging all genotypes carrying each of the three *GLU-D1* alleles, the lowest mean HMW/LMW ration was observed for the *Glu-D1l*, with no significant differences between *Glu-D1a* and *c* (Table 9). Comparisons of genotypes differing only in the alleles *Glu-D3b* and *c* (gt 5 and gt 8) showed no significant differences, although allele *b* exhibited a higher mean value than allele *c* when averaging across all genotypes (Table 7, Table 9).

Table 9: Mean and standard error of the quality tests averaging all genotypes carrying the *GLU-D1* and *GLU-D3* alleles.

Locus	Allele	SDSs (ml)	GPC (%)	HMW /LMW ratio	HMW-GS	LMW-GS
GLU-D1	<i>a</i>	27.01±1.61a	9.29±0.18b	0.25±0.0027a	3.14±0.05a	12.39±0.19a
	<i>c</i>	16.38±0.55c	9.11±0.11b	0.26±0.0046a	3.18±0.05a	12.43±0.19a
	<i>l</i>	19.46±0.23b	10.71±0.22a	0.21±0.004b	2.59±0.04b	12.14±0.36a
GLU-D3	<i>b</i>	17.25±0.6b	8.77±0.15b	0.26±0.0036a	3.06±0.04a	11.98±0.15a
	<i>c</i>	21.61±1.1a	9.76±0.15a	0.24±0.0039b	2.96±0.05a	12.42±0.17a

Means followed by different letters are significantly different at $P < 0.05$.

SDSS - SDS Sedimentation Test, GPC – Grain Protein Content, HMW - High Molecular Weight, LMW - Low Molecular Weight, GS - Glutenin Subunit

4.2.2.3. Effects of HMW and LMW genotype on HMW-GS and LMW-GS content

Mean HMW-GS content ranged from 2.59 (gt 1) to 3.64 (gt 9) among genotypes and from 3.88 ('Marius') to 5.35 ('Califa Sur') among varieties (Table 7). The genotype accounted for 51% of the variability. Similar to the HMW/LMW ratio, *GLU-D1*, *GLU-A3* and *GLU-B1* had significant effects, with the *GLU-D1* locus contributing the most variability, followed by *GLU-A3* and *GLU-B1* (Table 8).

Results from comparisons of gt 1, gt 2, and gt 3 (with *Glu-D1a*, *l* and *c*, respectively), gt 2 and gt 4 (both with *Glu-D1a*), gt 3, gt 9, and gt 7 (all with *Glu-D1c*) or gt 5 and gt 8 (with *Glu-D3b* and *c*, respectively) were consistent with those obtained for the HMW/LMW ratio. However, gt 4 had a lower HMW-GS content than gt 2 (Table 7). Averaging across genotypes carrying the same *GLU-D1* allele confirmed the negative effect of *Glu-D1l* on HMW-GS content. In contrast, *Glu-D1a* and *c* showed no significant differences as previously seen for the HMW/LMW ratio (Table 9). Similarly, *Glu-D3b* and *c* alleles exhibited comparable HMW-GS content (Table 9).

LMW-GS content ranged from 11.79 (gt 5) to 13.50 (gt 9) among genotypes and from 12.57 ('Berdun') to 15.86 ('Yecora') among varieties (Table 7). No significant effects of genotype or glutenin loci were detected for LMW-GS content (Table 8). Accordingly, differences among glutenin alleles or genotypes were not significant, except for the superiority of gt 9 relative to gt 6 and gt 5, all of which carried the *Glu-D1c* allele (Tables 6 and 7, Table 9)

4.2.3. Relationships between gluten quality and glutenin fractions

Regression analysis was performed to investigate the relationship between quality parameters (SDSs and GPC) and the quantities of different glutenin fractions (Table 10). The results indicated that, for the genotypes tested, only GPC showed a significant but weak relationship with SDSs. However, HMW/LMW ratio and HMW-GS amount accounted for around 25% of the variation in quality traits among genotypes differing only at the *GLU-D1* alleles (gt 1, gt 2, and gt 3). When focusing on genotypes with the *Glu-D1a* and *l* alleles (gt 1 and gt 2), SDSs was negatively influenced by GPC but strong and positively by HMW-GS content and HMW/LMW ratio. In contrast, when *Glu-D1l* allele (gt 1) was excluded, the analyses of *Glu-D1a* and *c* alleles (gt 2 and gt 3) revealed an increasing positive relation between SDSs and GPC ($R^2 = 0.80$). For genotypes carrying the *Glu-D1a* allele but differing at the *GLU-3* loci (gt 2 and gt 4), SDSs showed a positive association with GPC and HMW-GS content, but not relationships with the HMW/LMW ratio. In genotypes with the *Glu-D1c* allele and differing at *GLU-3* loci (gt 3, gt 7, and gt 9), the influence of GPC on SDSs was weaker, while the positive effects of HMW-GS content and the HMW/LMW ratio were more pronounced. No significant relationships were observed between SDSs and protein fractions in genotypes carrying the *Glu-D3b* and *c* alleles (gt 5 and gt 8).

Regarding GPC, a significant and negative association with HMW/LMW ratio was detected in the genotypes tested. This negative relationship persisted in genotypes differing only at the *GLU-D1* (gt 1, gt 2, and gt 3) but shifted to a positive association when the *Glu-D1l* allele (gt 1) was excluded (gt 2 and gt 3 results). A clear association between GPC and HMW-GS content was detected in genotypes carrying the *Glu-D1a* and *c* alleles (gt 2 and gt 3) ($R^2 = 0.30$) and in those with the *Glu-D1a* (gt 2 and gt 4) ($R^2 = 0.41$). It can be observed that the genotypes with *Glu-D1c* but differing at *GLU-3* (gt 3, gt 7, and gt 9) exhibited the strongest association of GPC with the HMW/LMW ratio and HMW-GS content ($R^2 = 0.31$ and 0.49 , respectively). As with SDSs, no significant associations between GPC and protein fractions were detected in genotypes carrying the *Glu-D3b* and *c* alleles (gt 5 and gt 8).

Table 10: Variation (coefficient of determination R²) of the SDSs values explained by GPC and glutenin fractions, and of GPC explained by glutenin fractions in the linear regression models performed using all the genotypes (gt) or genotypes grouped by glutenin composition (see Table 6). When the relationship was significant, the corresponding sign is indicated in brackets.

Model		Dependent variable	Explanatory variables			
gt	Allelic variation		GPC	HMW /LMW ratio ^a	HMW-GS	LMW-GS
All	<i>GLU-1, GLU-3</i>	SDSS	7 (+)	ns	ns	ns
		GPC	-	15 (-)	ns	ns
1, 2, 3	<i>Glu-D11,a,c</i>	SDSS	ns	27 (+)	24 (+)	ns
		GPC	-	18 (-)	9 (-)	ns
1, 2	<i>Glu-D11,a</i>	SDSS	28 (-)	73 (+)	76 (+)	ns
		GPC	-	35 (-)	ns	ns
2, 3	<i>Glu-D1a,c</i>	SDSS	80 (+)	26 (+)	25 (+)	ns
		GPC	-	21 (+)	30 (+)	ns
2, 4	<i>Glu-D1a, GLU-3</i>	SDSS	83 (+)	ns	27 (+)	ns
		GPC	-	ns	41 (+)	22 (+)
3, 7, 9	<i>Glu-D1c, GLU-3</i>	SDSS	51 (+)	20 (+)	45 (+)	ns
		GPC	-	31 (+)	49 (+)	ns
5, 8	<i>Glu-D3b,c</i>	SDSS	ns	ns	ns	ns
		GPC	-	ns	ns	ns

SDSS - SDS Sedimentation Test, GPC – Grain Protein Content, HMW - High Molecular Weight, LMW - Low Molecular Weight, GS - Glutenin Subunit ^a HMW -GS and LMW -GS values are expressed as those proteins ratio with respect to the band of 75KDa from the Precision Plus Protein Standards (BIORAD) included in all the gels
ns - Non-significant.

4.3.A new genetic map in durum wheat, allows the detection of QTL related to yield and grain protein content

4.3.1. Characterization of phenotypic traits

The phenotypic evaluation of the F_{4:7} durum wheat population was carried out over four growing seasons (2012-2013, 2013-2014, 2019-2020 and 2020–2021), including TKW, grain size and shape traits, flour color, and grain protein content (Table 11).

All traits were significantly different by season according to our ANOVA results (Table 11, Figure 16). TKW and grain size and shape traits were clearly

smaller in season 2012-2013, and higher in season 2013-2014 (Table 11, Figure 16). For both end-use quality related traits (b^* and GPC), the highest values were those of 2013-2014 and the lowest for 2019-2020 (Figure 16).

To determine whether the differences among seasons could be mainly attributed to environmental differences or genotype by environmental interactions also play a role, we explored the correlations between seasons for each trait (Figure 17). Ar, Perim and Majell showed significant positive correlations among all seasons, ranging between 0.83-0.37, 0.5-0.66 and 0.6-0.69, respectively. For GPC and TKW correlations were not significant between season 2012-2013 and 2013-2014, and for b^* , Minell and TKW neither were they between 2012-2013 and 2019-2020. However, the majority of the correlations were significantly positive (ranging between 0.24-0.47 (TKW), 0.21-0.51 (Minell), 0.25-0.4 (GPC), and 0.23-0.45 (b^*)), suggesting a strong genotypic effect.

Table 11: Mean values, standard deviation and range of yield, quality and grain size and shape traits evaluated for the F_{4:7} population in four seasons, differences tested by ANOVA (* p-value <0.05; ** p-value <0.01; *** p-value <0.001; ns: non-significant). Including TKW, four color (b*), grain protein content (GPC), and grain traits (grain area (Ar), grain perimeter (Perim), grain major ellipse (Majell), and grain minor ellipse (Minell)).

Trait	Season	Mean $\pm$ SD	Min	Max	ANOVA
Yield traits					
TKW	2012_2013	46.41 $\pm$ 4.21	39.08	57.70	***
	2013_2014	29.31 $\pm$ 2.68	23.25	37.88	
	2019_2020	37.58 $\pm$ 3.91	28.88	47.44	
	2020_2021	43.84 $\pm$ 3.33	32.53	57.67	
Quality traits					
Flour color	2012_2013	17.25 $\pm$ 0.88	15.32	19.26	***
	2013_2014	17.99 $\pm$ 0.67	15.97	19.46	
	2019_2020	17.34 $\pm$ 0.82	14.83	19.58	
	2020_2021	16.12 $\pm$ 0.84	14.39	18.30	
GPC	2012_2013	14.99 $\pm$ 1.59	12.04	19.70	***
	2013_2014	15.38 $\pm$ 1.17	13.03	18.81	
	2019_2020	14.01 $\pm$ 1.05	10.98	17.27	
	2020_2021	11.73 $\pm$ 1.19	9.42	16.91	
Grain size and shape traits					
Ar	2012_2013	20.37 $\pm$ 1.12	18.21	22.80	***
	2013_2014	15.87 $\pm$ 0.77	14.05	17.87	
	2019_2020	18.08 $\pm$ 0.98	16.06	20.30	
	2020_2021	18.79 $\pm$ 0.80	16.62	20.64	
Perim	2012_2013	23.16 $\pm$ 0.70	21.71	24.59	***
	2013_2014	20.62 $\pm$ 0.57	19.11	21.90	
	2019_2020	22.42 $\pm$ 0.73	20.75	24.15	
	2020_2021	22.33 $\pm$ 0.55	21.01	23.44	
Majell	2012_2013	7.80 $\pm$ 0.26	7.22	8.39	***
	2013_2014	7.03 $\pm$ 0.21	6.47	7.52	
	2019_2020	7.62 $\pm$ 0.27	6.94	8.21	
	2020_2021	7.47 $\pm$ 0.21	6.96	7.92	
Minell	2012_2013	3.33 $\pm$ 0.11	3.15	3.61	***
	2013_2014	2.87 $\pm$ 0.08	2.68	3.09	
	2019_2020	3.03 $\pm$ 0.09	2.84	3.26	
	2020_2021	3.21 $\pm$ 0.08	3.01	3.36	



Continue Figure 16

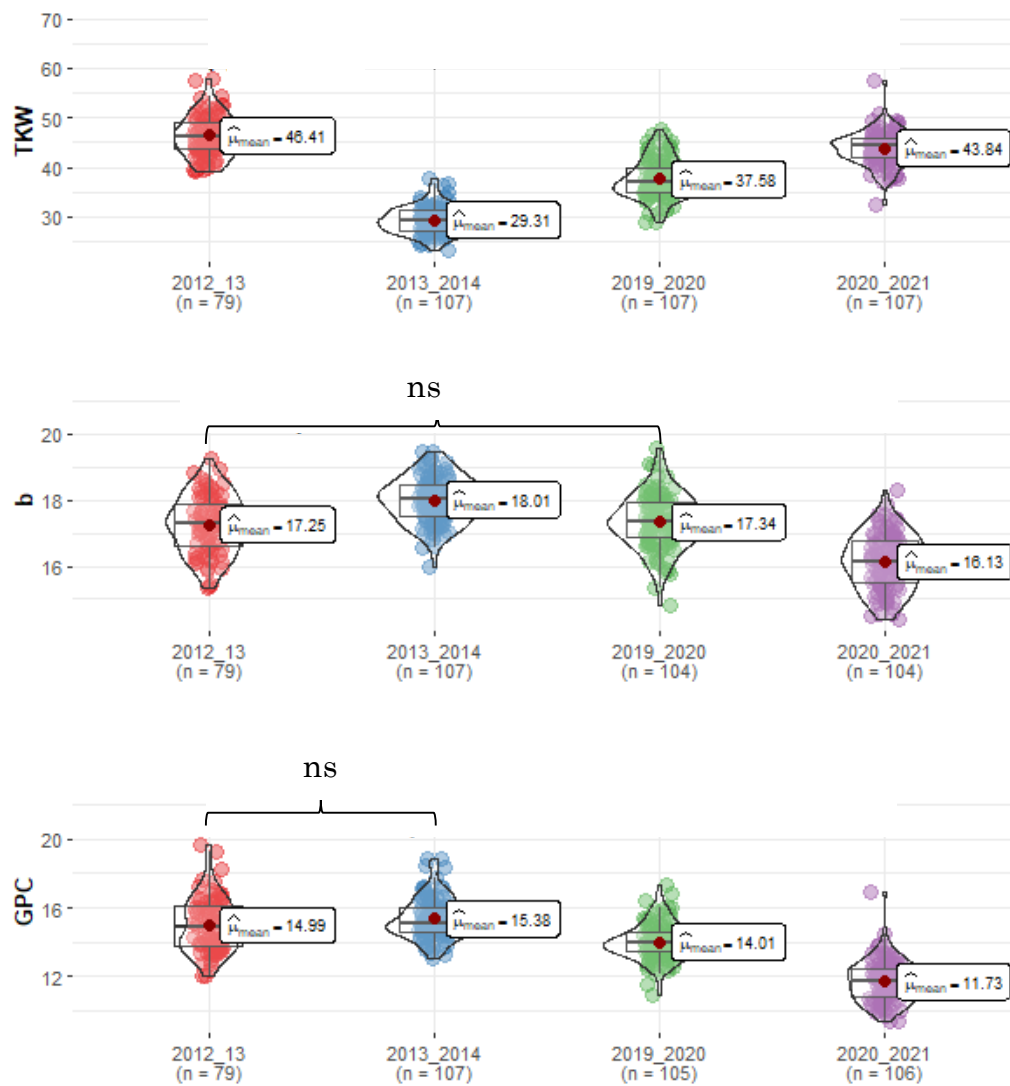


Figure 16: Distribution of the phenotypal values for each trait in each year.

Note: Significant differences between years tested by Games-Howell (indicated only non-significant ones p-value >0.05). Including TKW, flour color (b), grain protein content (GPC), and grain size and shape traits (grain area (Ar), grain perimeter (Perim), grain major ellipse (Majell), and grain minor ellipse (Minell)).

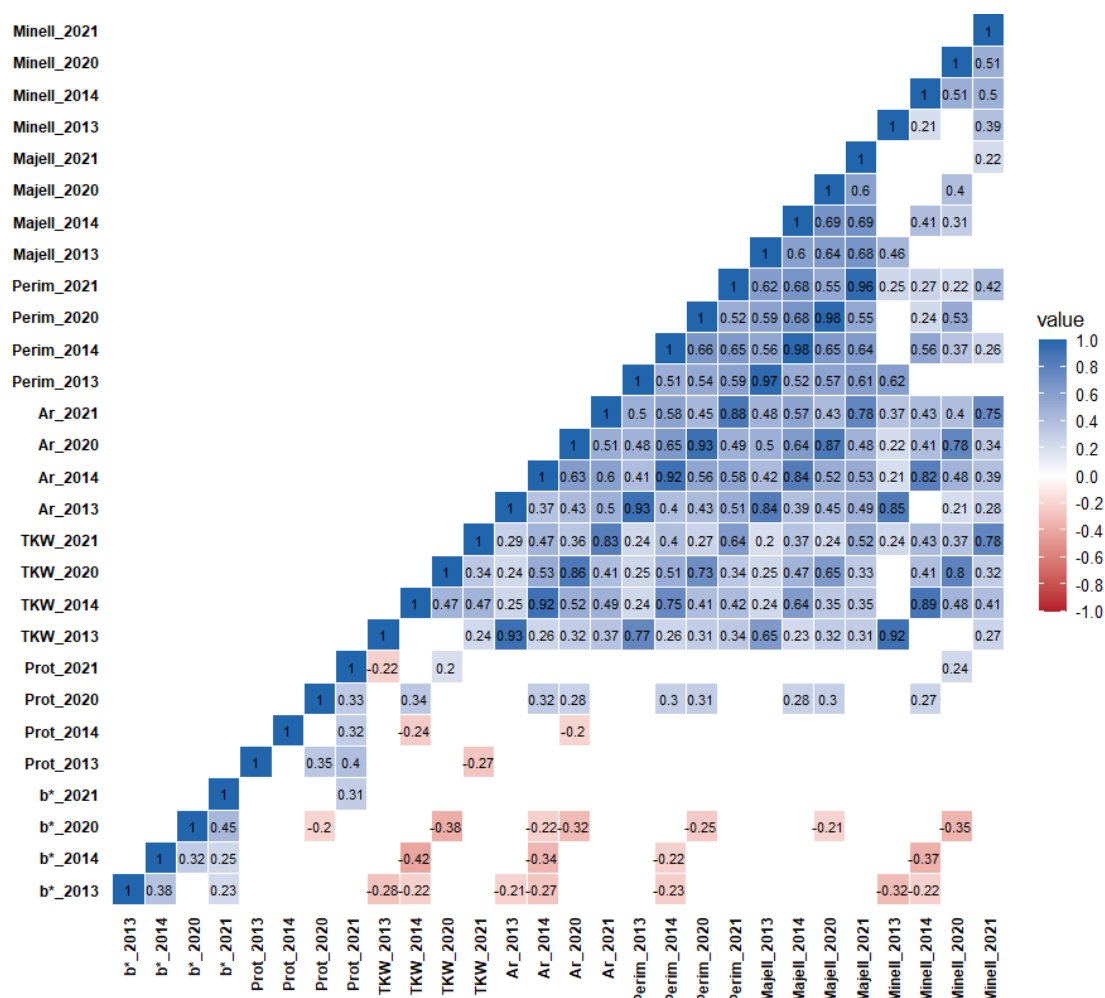


Figure 17: Heatmap of correlation between traits and seasons

Note: Only significant correlations are shown (p -value < 0.05). Including TKW, four color (b^*), grain protein content (Prot) and grain size and shape traits (grain area (Ar), grain perimeter (Perim), major grain ellipse (Majell), and grain minor ellipse (Minell)). The year included after the trait name corresponds to the harvest year for each season.

Then we analysed the correlations among the traits. As expected, TKW and grain size and shape traits were always positively correlated among them when values from the same season were compared. GPC and TKW were negatively correlated in season 2013-2014, showing no correlations in the rest of the trials. However, GPC was positively correlated with grain size and shape characteristics in 2019-2020. However, the color of the flour was negatively correlated with the rest of the traits in at least one year (Figure 17).

4.3.2. Genetic map

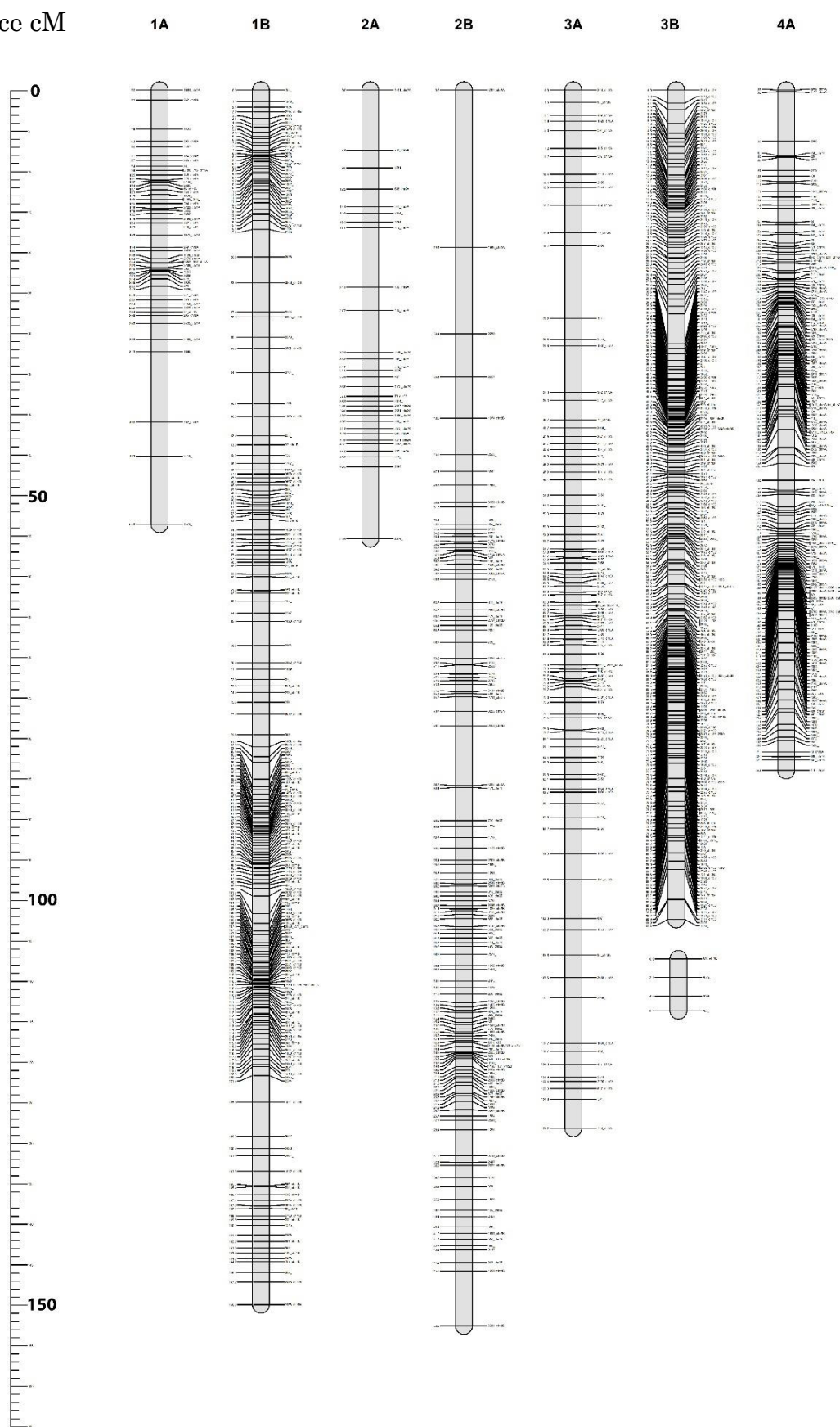
High-throughput genotyping rendered a total of 143225 PAV markers (presence / absence) and 52511 SNP markers (single polymorphic nucleotide). After performing the filtering to select polymorphic high-quality markers for genetic map construction. There were 1573 PAV and 639 SNP markers remained. Markers were grouped into 31 linkage groups based on LOD score that were assigned to the 14 durum wheat chromosomes based on the physical position of each marker, when available. Within each group, genetic markers were ordered by regression mapping, and genetic distances were estimated with Kosambi (1944). When the genetic distances between adjacent markers did not allow for a proper arrangement, we split the chromosome into different linkage groups. Finally, our map included 17 linkage groups with chromosomes 3B, 5A, and 5B divided in two linkage groups (Figure 18).

The final map included a total of 2117 markers (Table 12), which allowed us to assign 1027 markers for which the physical position was not determined by blast analysis, and 67 located in the unknown chromosome. Regarding genetic distances our map spanned 1471.1cM, with chromosome 2B being the longest, and 1A the shortest. The average distance between markers was 0.87cM with a maximum of 10.11cM (excluding the chromosomes with two linkage groups) (Table 12).

Table 12: Description of the estimated genetic map for F_{4:7} Endural X Aldura population.

Chr	Marker nb		Mean distance cM		Max distance cM	
	A	B	A	B	A	B
1	53	218	1.0	0.7	8.7	4.2
2	30	151	1.9	1	8.9	19.4
3	102	271	1.3	0.4	9	-----
4	180	98	0.5	0.8	6.1	12.9
5	90	121	1.6	1	-----	-----
6	233	205	0.6	0.4	9.7	3.3
7	184	181	0.6	0.5	23.6	3.7

Distance cM



Continue Figure 18

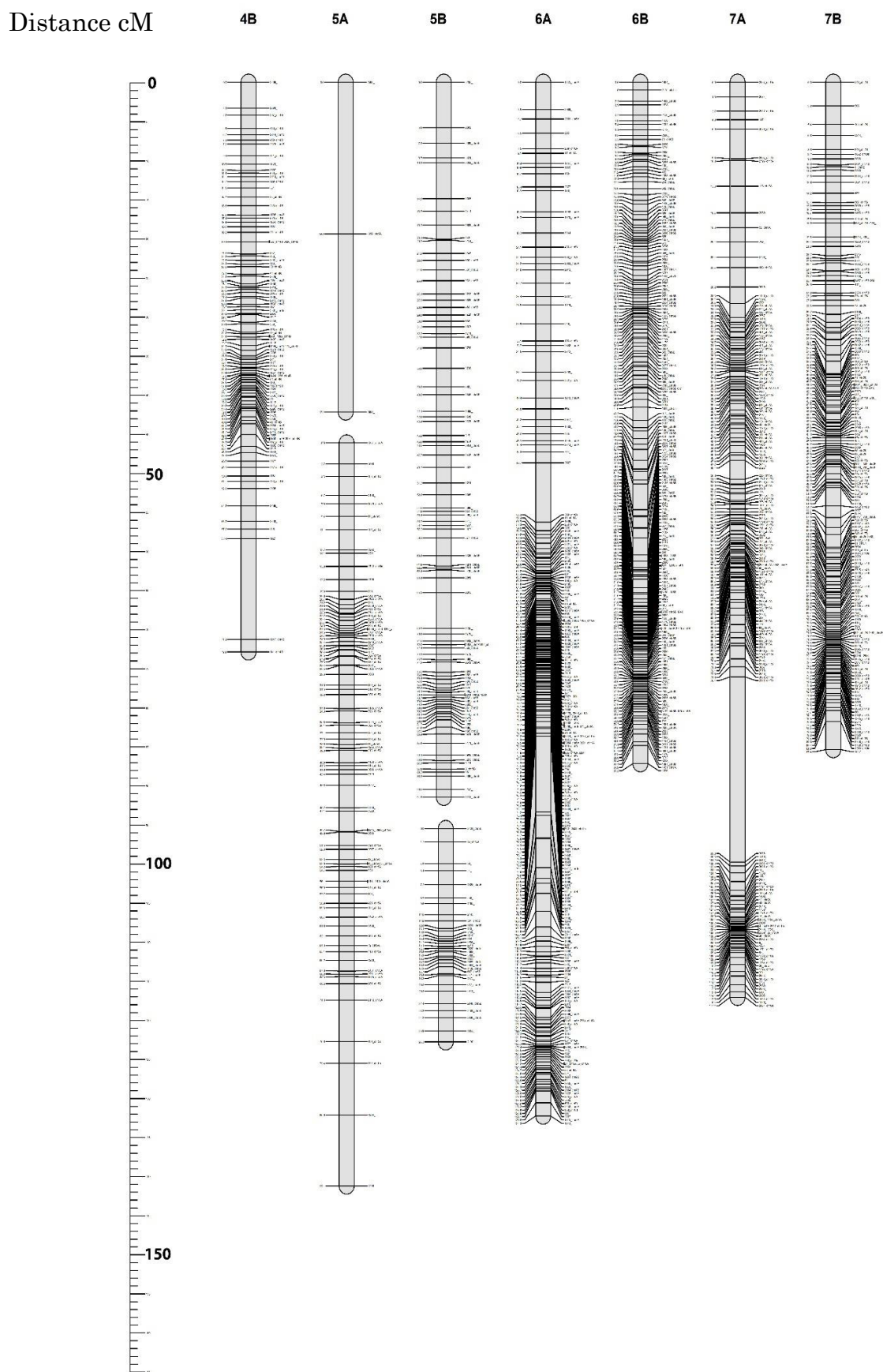


Figure 18: Genetic map estimated for the F_{4:7} Endural X Aldura population.

We then explored the correspondence between our genetic map and the physical position of the markers in the reference genome (Figure 19, Annex 2). This allowed us to detect recombination hotspots (as example Figure 19 chromosome 1B), possible rearrangements with respect to Svevo or possible errors in genome assembly (as example Figure 19 chromosomes 6A and 7A), and lack of coverage in our map (as example Figure 19 chromosome 2A).

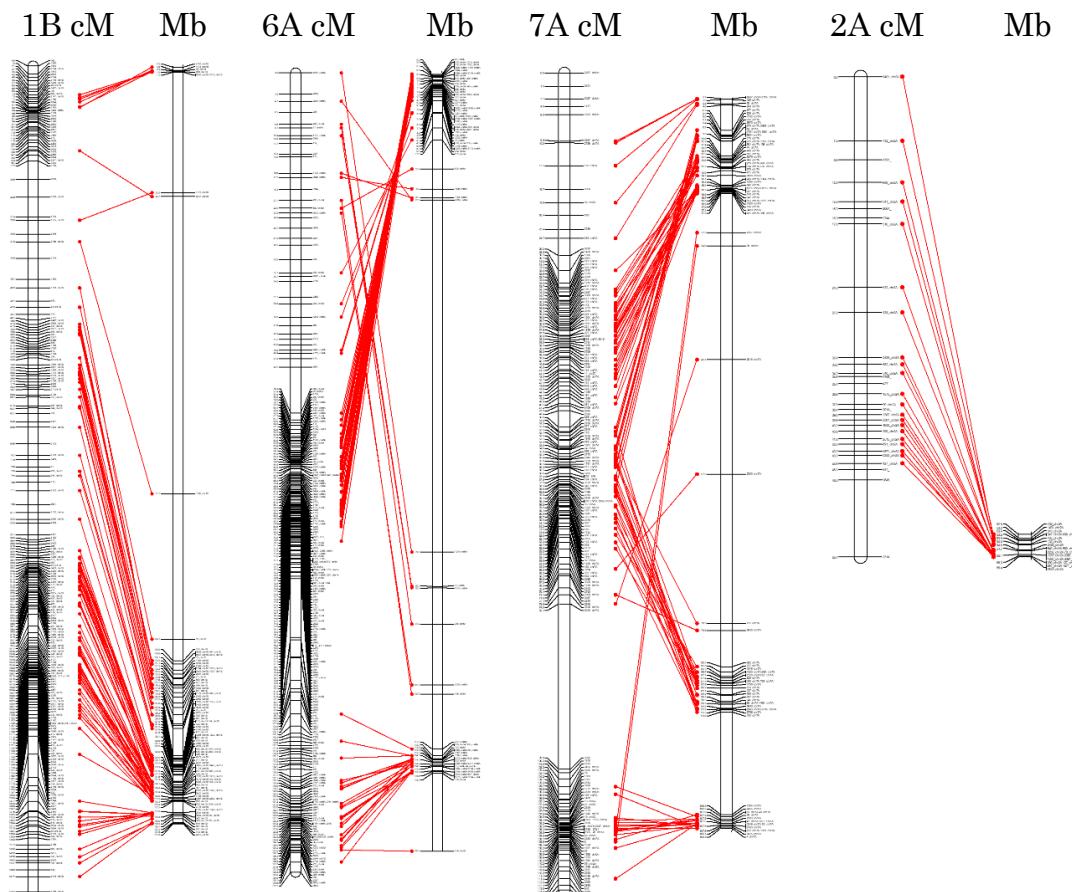


Figure 19: Comparison between the genetic map and the physical location in the reference genome.

Note: Selection of chromosomes 1B, 6A, 7A, and 2A.

4.3.3. QTL detection.

The QTL analysis allowed the identification of genomic regions associated with TKW, GPC, flour color (b *) and grain size and shape traits (Figure 20, Annex 3). The identified QTLs were mapped to 10 chromosomes (1B, 2B, 3A, 3B, 4A, 4B, 5B, 6B, 7A, 7B), with varying degrees of stability and pleiotropic effects.

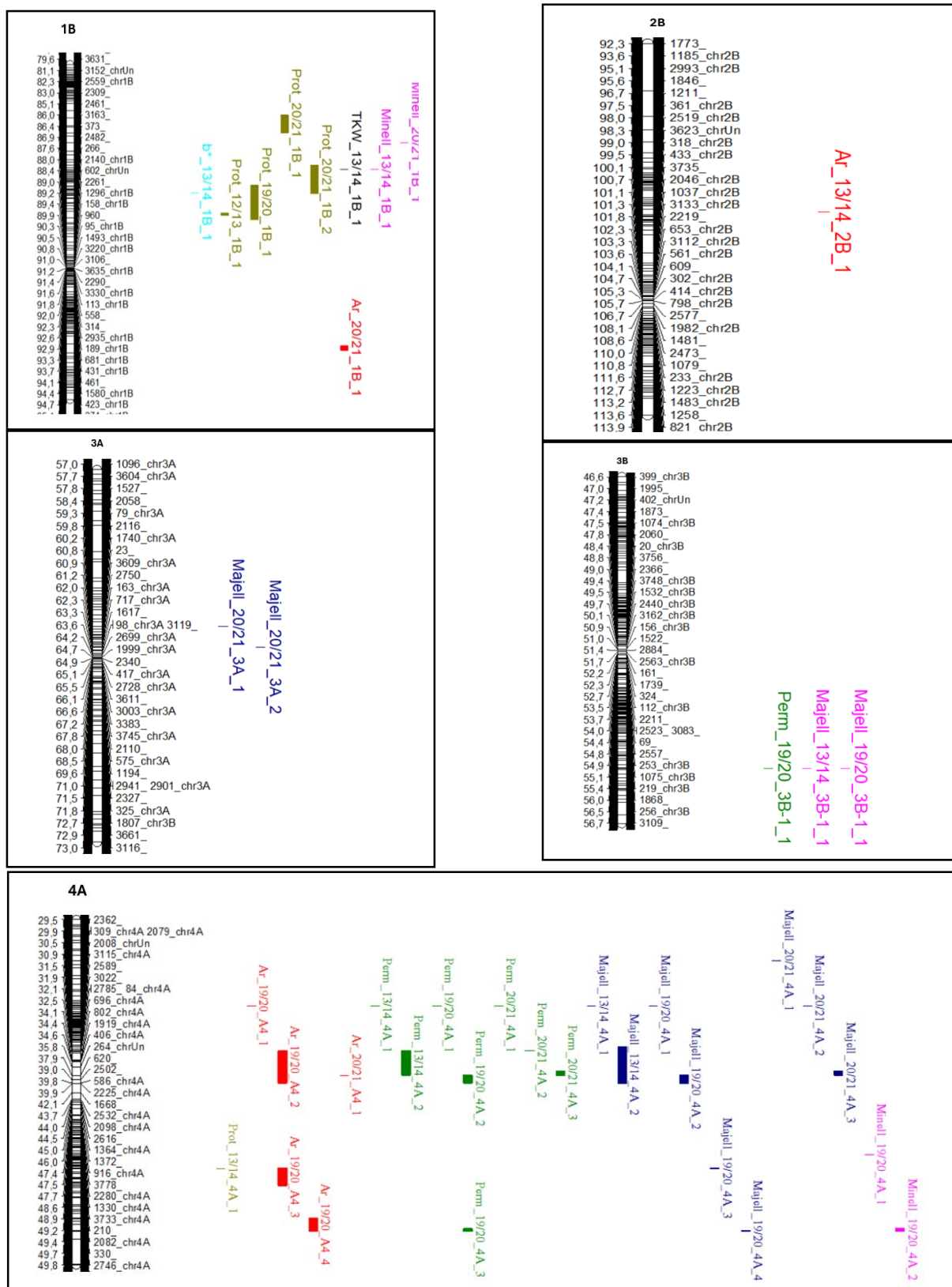
Associations with TKW were detected on chromosomes 1B and 4B. However, none of them were stable. Although, the QTL located on chromosome 4B (10.3-11.6cM) overlapped with other QTLs controlling grain size and shape traits (Figure 20).

Regarding grain size and shape traits, QTLs were identified on chromosomes 1B, 2B, 3A, 3B, 4A, 4B, 5B, 6B, and 7B (Figure 20, Annex 3). Chromosomes 4A (21cM, 30.9-39.7cM) and 4B (9.3-29.6cM) harboured overlapping QTLs for several grain traits, stable across different seasons (Table 13). Although chromosome 3B included a QTL for Majell, and chromosome 6B one for Perim.

The identification of stable QTLs, particularly for flour color on chromosome 7A, GPC on chromosome 1B, and grain-related traits on chromosomes 4A and 4B, highlights the importance of those genomic regions (Table 13).

Table 13. Summary of stable QTLs. Including the name of overlapping QTLs, the chromosome, start and finish positions in cM and Mb (when available), and maximum LOD score.

QTLs	Chr	Start		Finish		LOD max
		Pos cM	Pos Mb	Pos cM	Pos Mb	
Ar_4B_1 Ar_20/21_4B_1 Ar_13/14_4B_1 Ar_19/20_4B_1 Ar_19/20_4B_2	4B	5.878	61,72	29.61	-----	4.86
Ar_4A_2 Ar19/20_4A_2 Ar_20/21_4A_1	4A	31.854	-----	39.773	631.6	4.84
Majell_4A_1 Majell_13/14_4A_1 Majell_19/20_4A_1 Majell_20/21_4A_2	4A	21.039	-----	21.039	-----	5.09
Majell_4A_2 Majell_13/14_4A_2 Majell_20/21_4A_3 Majell_19/20_4A_2	4A	30.922	624,51	39.773	631.6	5.09
Majell_3B_1 Majell_19/20_3B_1_1 Majell_13/14_3B_1_1	3B_1	86.125	80,95	86.125	81.0	3.06
Minell_4B_1 Minell_19/20_4B_1 Minell_20/21_4B_2 Minell_13/14_4B_1	4B	9.356	54,86	14.661	35.80	3.30
Perim_4A_1 Perim_13/14_4A_1 Perim_19/20_4A_1 Perim_20/21_4A_1	4A	21.039	-----	21.039	-----	4.23
Perim_4A_2 Perim_19/20_4A_2 Perim_13/14_4A_2 Perim_20/21_4A_2 Perim_20/21_4A_3	4A	31.854	-----	39.773	631.55	3.91
Perim_6B_1 Perim_19/20_6B_2 Perim_13/14_6B_1	6B	44.232	16,08	44.569	16.2	3.26
Perim_4B_1 Perim_20/21_4B_1 Perim_13/14_4B_1 Perim_13/14_4B_2	4B	10.356	-----	29.61	-----	5.80
GPC_1B_1 Prot_19/20_1B_1 Prot_12/13/1B_1 Prot_20/21_1B_2	1B	54.014	548,45	69.63	-----	5.57
b*_7A_1 b*_20/21_7A_1 b*_13/14_7A_1 b*_19/20_7A_1	7A	86.146	275,79	117.136	287.61	8.69



Continue Figure 20.

Note: flour color b* (light blue), GPC (prot, light green), TKW (black), Ar (red), Perim (green), Majell (blue), Minell (pink).

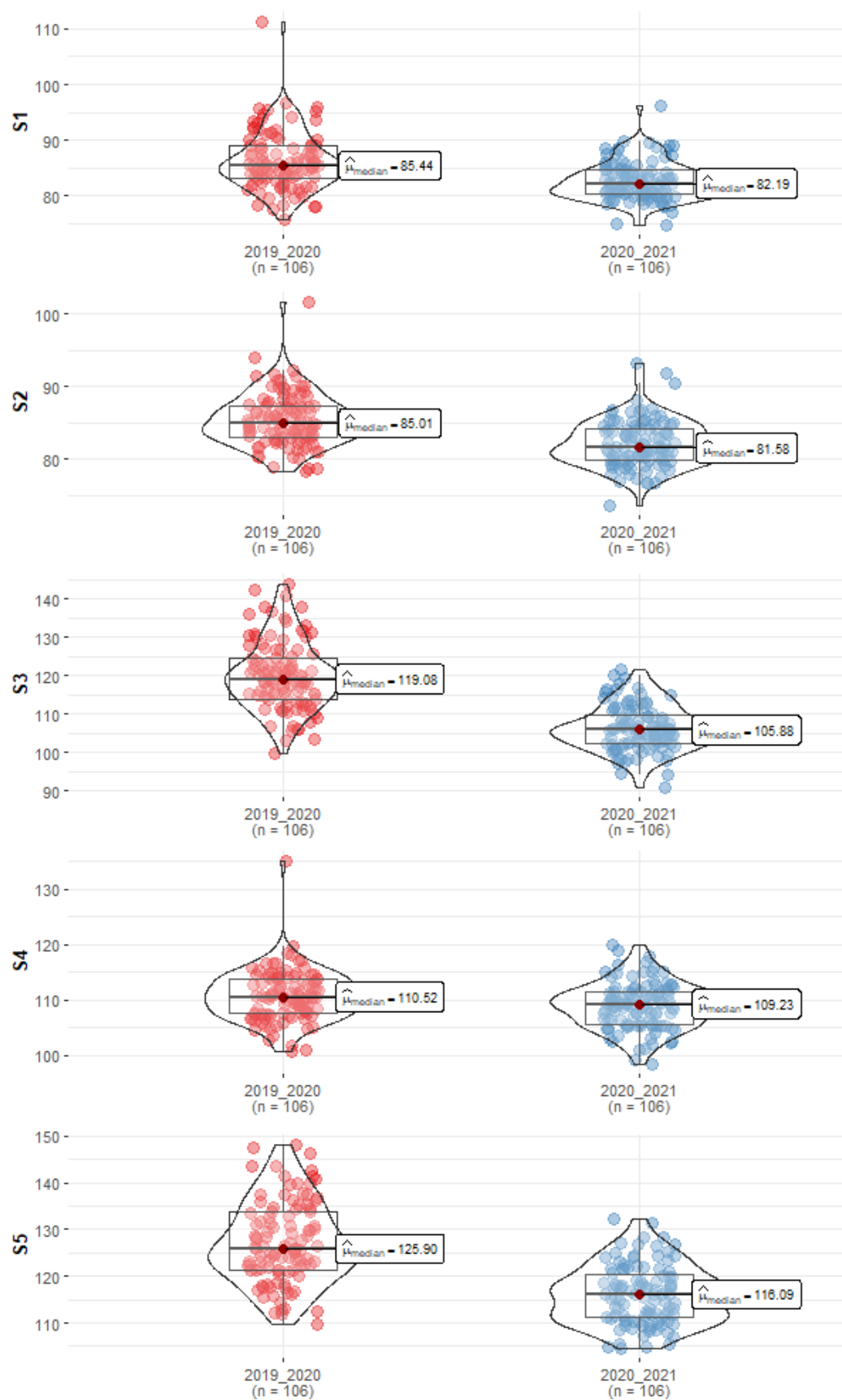
4.4. Identification of genetic factors controlling rheological properties in durum wheat by QTL detection

4.4.1. Phenotypic characterization

The phenotypic evaluation of the F_{4:7} durum wheat population was carried out over two growing seasons (2019-2020 and 2020–2021), including quantification of HMW-GS, LMW-Gs and HMW-GS to LMW-GS ratio (HMW/LMW), gluten strength (SDSs), and percentage of SRC for water (S1), alkaline water (S2), lactic acid (S3), sodium carbonate (S4), and sucrose (S5) (Table 14).

Table 14: Mean values, standard deviation and range of quality traits evaluated for the F_{4:7} population in two different seasons. Differences among seasons compared by Mann-Witney test (* p-value <0.05; ** p-value <0.01; *** p-value <0.001; ns: non-significant).

Trait	Season	Mean $\pm$ SD	Min	Max	Endural	Aldura	Mann-Witney
HMW/LMW	19/20	0.1434 $\pm$ 0.0127	0.1162	0.1834	0.14	0.14	***
	20/21	0.1307 $\pm$ 0.0161	0.1097	0.2051	0.13	0.13	
HMW-GS	19/20	1.0152 $\pm$ 0.143	0.7871	1.4744	1	0.98	ns
	20/21	1.0654 $\pm$ 0.221	0.7342	1.9534	1	0.97	
LMW-GS	19/20	1.0004 $\pm$ 0.117	0.7354	1.3892	1	0.99	***
	20/21	1.0726 $\pm$ 0.142	0.7753	1.4622	1	1.01	
SDSs (mm)	19/20	26.94 $\pm$ 7.908	19.00	45.00	23.5	23.5	ns
	20/21	27.67 $\pm$ 10.084	18.00	54.00	21.5	21.5	
S1 (%)	19/20	86.24 $\pm$ 5.190	75.82	111.23	81	84.7	***
	20/21	82.64 $\pm$ 3.402	74.82	96.13	78.5	76.2	
S2 (%)	19/20	85.42 $\pm$ 3.632	78.22	101.64	86	85.7	***
	20/21	82.13 $\pm$ 3.223	73.69	93.23	79	80.2	
S3 (%)	19/20	120.01 $\pm$ 8.920	99.64	143.70	120	109.7	***
	20/21	106.27 $\pm$ 5.808	91.04	121.58	119.5	107.2	
S4 (%)	19/20	110.07 $\pm$ 4.596	100.7	135.0	112.3	109	**
	20/21	108.93 $\pm$ 4.153	98.47	119.79	100.8	105.5	
S5 (%)	19/20	127.2 $\pm$ 8.670	109.7	148.1	112	123.5	***
	20/21	116.1 $\pm$ 6.253	104.7	132.3	113.4	109.7	



Continue Figure 21



Figure 21: Distribution of the phenotypic values for each trait in each year
Note: Significant differences between years are indicated. Including quantification of HMW-GS, LMW-GS and HMW-GS to LMW-GS ratio (HMW/LMW), gluten strength (SDSs), and percentage of SRC for water (S1), alkaline water (S2), lactic acid (S3), sodium carbonate (S4) and sucrose (S5).

All traits, except HMW-GS quantity and SDSs, were significantly different by season according to our results. Values were higher for LMW-GS quantity, and lower for all SRC in 2020-2021 (Table 14, Figure 21). The most notable reductions were observed in lactic acid (S3, from 120.01 to 106.27) and sucrose (S5, from 127.2 to 116.1),

To determine whether the differences among seasons could be mainly attributed to environmental differences or if genotype by environmental interactions also play a role, we explored the correlations between seasons for each trait (Figure 22). All traits were significantly correlated. Correlations were highly positive for SDSs, lactic acid (S3), and sucrose (S5) (0.9, 0.53, 0.57, respectively); positive for water (S1), alkaline water (S2), and sodium carbonate (S4) (0.41, 0.45, 0.41, respectively); and weak positive for prolamin quantification traits (0.27, 0.22, 0.27 for HMW/LMW ratio, HMW-GS, LMW-GS, respectively).

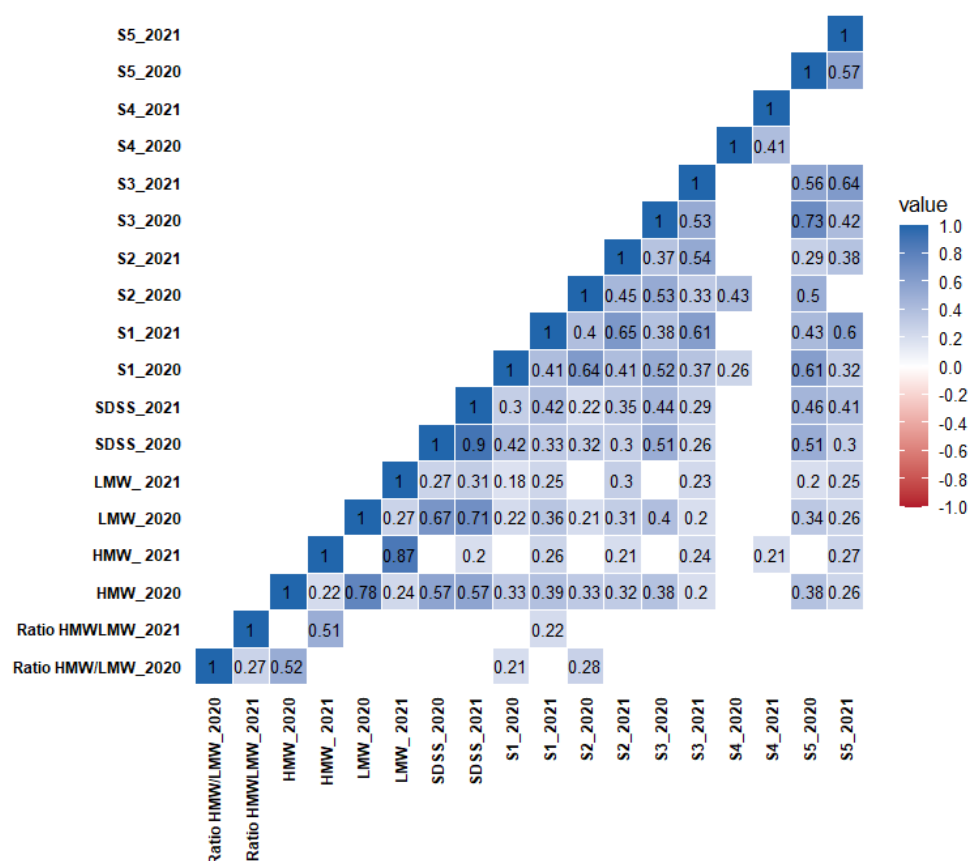


Figure 22: Heatmap of correlation between traits and seasons

Note: Only significant correlations are shown (p -value < 0.05). Including quantification of HMW-GS, LMW-GS and HMW-GS to LMW-GS ratio (HMW/LMW), gluten strength (SDSs), and percentage of SRC for water (S1), alkaline water (S2), lactic acid (S3), sodium carbonate (S4) and sucrose (S5). The year included after the trait name corresponds to the harvest year for each season.

Regarding correlations among different traits, sodium carbonate SRC values were only weakly correlated with HMW-GS and water SRC in the season 2020-2021, and positively correlated with alkaline water SRC (S2). The rest of the traits were all positively correlated between them in each season. The greater correlations were found between HMW-GS and LMW-GS (0.78 and 0.87 for the two seasons) (Figure 22). Moreover, SDSs was also highly correlated ($r^2 > 0.5$) with prolamins quantification traits, S3 and S5 in 2019-2020. The different SRC also showed positive correlations among them, ranging between 0.38-0.73, except for sodium carbonate (S4).

4.4.2. Glutenin and gliadin alleles genotyping and Influence of prolamins loci on end-use quality traits.

The segregating population genotyping for prolamins revealed a close to 1:1 segregation for all the loci (Table 15). The heterogeneous $F_{4:7}$ were not included in the analysis employing the different loci as factor.

Table 15: Allelic composition of glutenin and gliadin polymorphic loci, in the parents and in the population with respect to the parent alleles. Ht (heterogeneous), no data (genotyping failed).

Loci	Endural	Aldura	Population			
			Endural	Aldura	Ht	no data
<i>GLU-B1</i>	<i>e</i> (20x+20y)	<i>d</i> (6+8)	44	57	5	1
<i>GLU-A3</i>	<i>l</i> (6+10)	<i>k</i> (5)	48	55	0	4
<i>GLU-B3</i>	<i>w</i> (2+4+15+17)	<i>s</i> (8+9+13+16)	51	51	4	1
<i>GLU-B2</i>	<i>a</i> (12)	<i>b</i> (nula)	47	53	0	7
<i>GLI-B1</i>	<i>c</i> (γ-45)	<i>a</i> (γ-42)	52	51	2	4
<i>GLI-A2</i>	α (α-block+α-10)	α (α-block+α-11)	60	46	0	1
<i>GLI-B2</i>	γ-1+δ-4	γ-2+δ-3+δ-5	55	51	0	1

Subsequently, we explored the influence of each gene over the analyzed traits (Table 16). Regarding gliadins, no association was found between *GLI-B2*, the only polymorphic locus located outside the homoeologous group 1, and any of the traits. *GLI-A1* was only significantly associated with water SRC (S1) in one

season. However, *GLI-B1* was associated with all the traits except HMW to LMW ratio and sodium carbonate SRC (S4). It must be noted that, this might be due its genetic linkage with the LMW-GS locus *GLU-B3*, that rendered almost the same results. If we focus in the rest of polymorphic LMW-GS loci, *GLU-B2* was associated with all the traits, except HMW-GS to LMW-GS ratio in at least one season, and *GLU-A3* was associated with all the traits except HMW-GS to LMW-GS ratio and S4. The only polymorphic HMW-GS locus was associated with S3, S5 HMW-GS, and LMW-GS in 2019-2020, and with SDSs in both seasons.

From another perspective it is clear that, the trait with most significant associations was SDSs, followed by S5 and S3

Table 16: Association between prolamin loci and end-use quality traits by season. Including quantification of HMW-GS, LMW-GS and HMW-GS to LMW-GS ratio (HMW/LMW), gluten strength (SDSs), and percentage of SRC for water (S1), alkaline water (S2), lactic acid (S3), sodium carbonate (S4) and sucrose (S5).

Loci	Season	S1	S2	S3	S4	S5	SDSs	HMW	LMW	HMW/LMW
<i>GLU-A3</i>	19-20	ns	ns	**	ns	**	***	***	***	ns
	20-21	*	*	**	ns	***	**	ns	ns	ns
<i>GLU-B1</i>	19-20	ns	ns	**	ns	**	***	***	***	ns
	20-21	ns	ns	ns	ns	ns	***	ns	ns	ns
<i>GLU-B2</i>	19-20	ns	ns	***	ns	***	***	***	***	ns
	20-21	***	*	***	*	***	***	**	***	ns
<i>GLU-B3</i>	19-20	**	ns	***	ns	***	***	***	***	ns
	20-21	***	**	***	ns	***	***	ns	**	ns
<i>GLI-A2</i>	19-20	ns	ns	ns	ns	ns	ns	ns	ns	ns
	20-21	*	ns	ns	ns	ns	ns	ns	ns	ns
<i>GLI-B1</i>	19-20	**	ns	***	ns	***	***	***	***	ns
	20-21	***	*	***	ns	***	***	ns	**	ns
<i>GLI-B2</i>	19-20	ns	ns	ns	ns	ns	ns	ns	ns	ns
	20-21	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns (not significant, $p > 0.05$), * ($0.05 > p > 0.01$), ** ($0.01 > p > 0.001$), *** ($p < 0.001$).

4.4.3. QTL detection.

The QTL analysis allowed the identification of genomic regions associated with HMW-GS and LMW-GS quantity, HMW-GS to LMW-GS ratio, SDSs, and SRC for all the five different solvents. The identified QTLs were mapped to 10 chromosomes (1B, 3A, 3B, 4A, 4B, 5B, 6A, 6B, 7A, 7B), with varying degrees of stability and pleiotropic effects (Table 17, Figure 23, Annex 4, Annex 5).

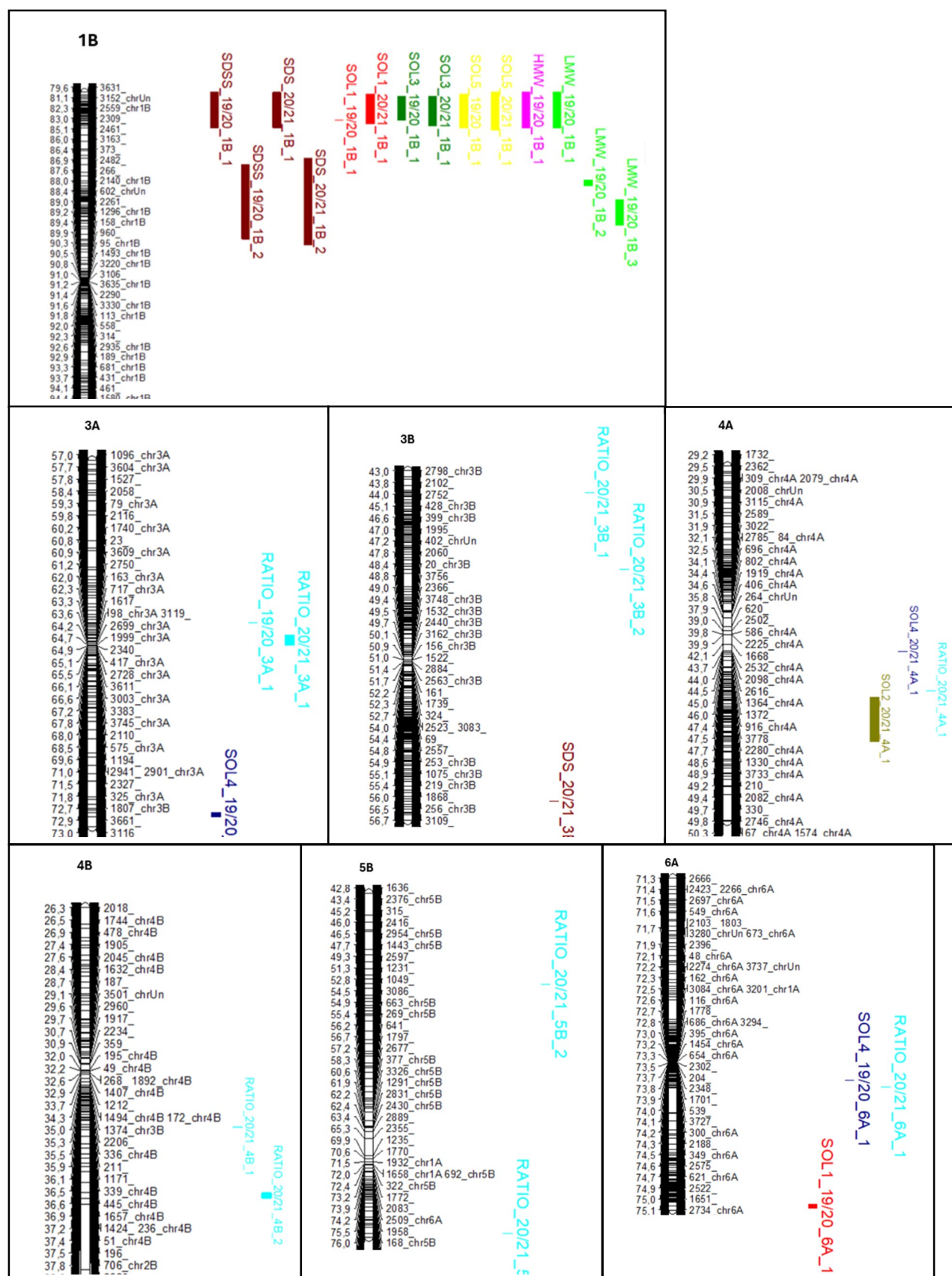
Associations with HMW-GS and LMW-GS were detected in chromosome 1B, however only in 2019-2020. HMW-GS to LMW-GS ratio was associated with regions in seven chromosomes (3A, 3B, 4A, 4B, 5B, 6A, 7B), but only in one season. Similarly, alkaline water (S2) and sodium carbonate (4) SRC were associated with regions in 4A, 7A or 3A, 4A, 6A, 7A chromosomes respectively, but none of them were stable (Figure 23).

For water (S1), acid lactic (S3), and sucrose (S5) SRC plus SDSs stable associations were detected in chromosome 1B between 0 and 18cM, overlapping with glutenins coding loci. Those results are in accordance with the associations described in table 16, with different glutenin loci. For SCR traits no stable associations were found (Figure 23, Table 17). For SDSs two stable associations were also detected in chromosome 6B at 38 and 49 cM (Table 17).

Table 17: Summary of stable QTLs. Including the name of overlapping QTLs, the chromosome, start and finish positions in cM and Mb (when available), and maximum LOD score or K value.

QTL	Chr	Start		Finish		LOD/K max
		Pos cM	Pos Mb	Pos cM	Pos Mb	
S1_1B_1	1B	1	-----	15.164	-----	4.46
S1_20/21_1B_1						
S1_19/20_1B_1						
S3_1B_1	1B	2.053	-----	16.142	-----	4.89
S3_19/20_1B_1						
S3_20/21_1B_1						
S5_1B_1	1B	0	-----	18.236	-----	10.00
S5_20/21_1B_1						
S5_19/20_1B_1						
SDSs_1B_1	1B	0	-----	17.236	-----	46.267
SDS_19/20_1B_1						
SDS_20/21_1B_1						
SDSs_1B_2	1B	31.921	383.65	73.552	589.60	52.221
SDS_20/21_1B_2						
SDS_19/20_1B_2						
SDSs_6B_1	6B	38.671	18.00	39.128	-----	9.841
SDS_19/20_6B_1						
SDS_20/21_6B_1						
SDSs_6B_2	6B	49.802	44.46	49.802	44.46	9.841
SDS_19/20_6B_2						
SDS_20/21_6B_2						

Finally, it must be noted that chromosome 1A, also polymorphic for *GLU-A3* was not associated with any of the traits. However, chromosome 1B, polymorphic for *GLU-B1*, *GLU-B2* and *GLU-B3* was associated with S1, S3, S5 SRC, SDSs, HMW-GS and LMW-GS quantity, being the chromosome with more associations (Figure 23, Annex 4, Annex 5).



Continue Figure 23

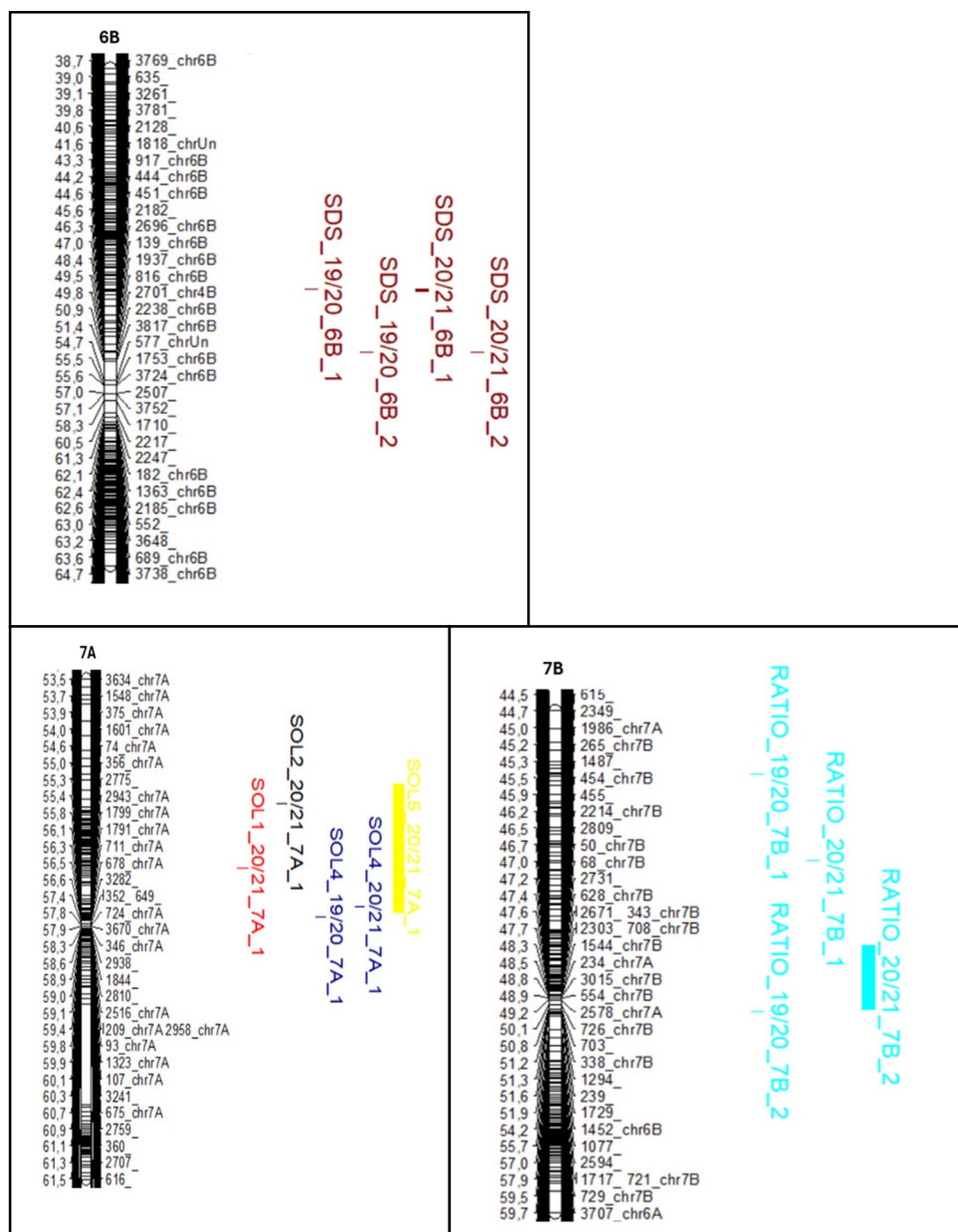


Figure 23: QTLs detected along the different seasons

Note: HMW-GS, LMW-GS and HMW-GS to LMW-GS ratio (RATIO), gluten strength (SDSS), and percentage of SRC for water (SOL1), alkaline water (SOL2), lactic acid (SOL3), sodium carbonate (SOL4) and sucrose (SOL5)

4.5. Investigating *Phytoene Synthase 1* and *Lipoxygenase 1* Effects on Wheat Flour Color: Clues for Breeding High-Quality Durum Wheat

4.5.1. Endural and Aldura cultivars, with contrasting phenotypic values, are polymorphic for one carotenoid synthesis and one catalysis gene.

The cultivars Endural and Aldura were characterized in two different seasons for whole flour yellow color (b^*), whole flour yellow pigment content (YPC), dough yellow pigment content (YPCd) and yellow pigment loss (YPL %), showing differences in all the traits (Table 18). Aldura presented higher whole flour color and YPC. However, differences between both parents were reduced in dough (YPCd), as yellow pigment was degraded in Aldura (YPL%=12.49% and 10.32 % for 2019-2020 and 2020-2021) but not in Endural (YPL%=0 both seasons).

We screened parental lines for four carotenoids biosynthetic pathway (*PSY1-A1*, *PSY1-B1*, *PDSB* and *ZDSA*) and two carotenoid catalysis (*LPX-B1.1* and *LPX-A3*) genes. Both cultivars carried the same allele for four of the genes *PSY1-B1o*, *PDSB*, *ZDSA* and *LPX-A3* (allele UC1113), being only polymorphic for *PSY1-A1* and *LPX-B1.1*. Aldura carries the alleles *Psy1-A1l* and *LPX-B1.1a*, while Endural carries *Psy1-A1a* and *LPX-B1.1c*.

Table 18: Phenotypic characterization, including b^* value from the colorimeter, yellow pigment content (YPC), yellow pigment content in dough (YPCd) and percentage of yellow pigment loss (YPL%). Endural and Aldura values, plus segregating population average $\pm$ standard error and range (minimum and maximum) are indicated. r^2 corresponds to Spearman correlation value for each trait between seasons.

Season		b^*	YPC (mg β -car)	YPCd (mg β -car)	YPL %
2019-2020	Endural	16.47	9.31	9.46	0%
	Aldura	19.38	11.60	10.15	12.49%
	Pop mean$\pm$se	17.33 $\pm$ 0.08	9.77 $\pm$ 0.16	9.41 $\pm$ 0.17	6.32 $\pm$ 0.74
	Pop min-max	14.83-19.58	6.02-13.48	5.87-13.81	0%-34.8%
2020-2021	Endural	14.54	6.91	7.14	0%
	Aldura	17.98	10.69	9.59	10.32%
	Pop mean$\pm$SE	16.14 $\pm$ 0.08	9.25 $\pm$ 0.12	8.14 $\pm$ 0.15	13.36 $\pm$ 1.18
	Pop min-max	14.39-18.30	7.12-13.09	5.18-11.93	0%-43.78%
r^2		0.43***	0.53***	0.59***	0.58***

* p-value <0.05; ** p-value <0.01; *** p-value <0.001; ns: non-significant.

4.5.2. The population characterization reveals the key role of the genotype on phenotypical values

A population of 104 advanced lines derived from Endural and Aldura was also characterized in two seasons. At phenotypical level all the traits (b^* , YPC, YPCd and YPL %) present a positive significant correlation among seasons, ranging from 0.43 for b^* to 0.59 for YPCd, showing the key role of the genotype (Table 18). However, as with the parent cultivars, the average population values for flour yellow color, yellow pigment content, and yellow pigment content in dough were significantly different, being higher for season 2019-2020. Regarding YPL %, the values were also significantly different between seasons, but in this case YPL % was higher in 2020-2021 than in 2021-2020 (13.36%, and 6.32 % respectively) (Table 18, Table 19).

The correlation among traits revealed positive significant correlations between b^* , YPC and YPCd (ranging between 0.46-0.79 and 0.3-0.48 for seasons 2019-2020 and 2020-2021 respectively) but negative correlations between YPCd and YPL % (-0.43 and -0.75, for seasons 2019-2020 and 2020-2021 respectively). No significant correlations were found between the flour color (b^*) or yellow pigment content (YPC) and YPL %, which suggest that carotenoid content does not influence the carotenoid catabolic pathway (Figure 24).

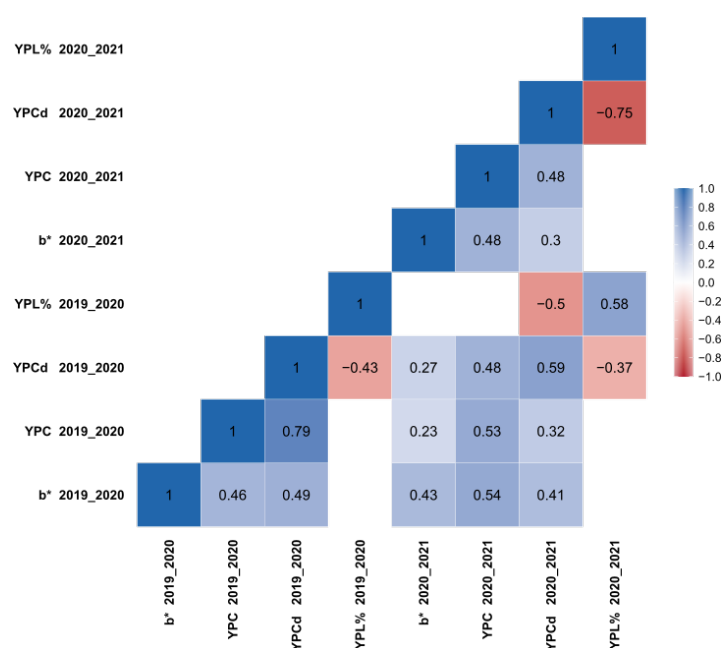


Figure 24: Heatmap of correlation between traits and seasons

Note: Only significant correlations are shown (p-value < 0.05). Including b^* value from the colorimeter, yellow pigment content (YPC), yellow pigment content in dough (YPCd) and percentage of yellow pigment loss (YPL%).

Table 19: Phenotypic data by genotype, including min-max, average $\pm$ SD, plus ANOVA significance results, for significant factors the percentage of variance (η^2) explained estimated as sum of squares between groups by the sum of squares total is indicated.

		<i>PSY-A1</i>		<i>LPX-B1.1</i>		ANOVA						
alleles		<i>aa</i>	<i>ll</i>	<i>aa, ac</i>	<i>cc</i>	factor	<i>PSY1-A1</i>	<i>LPX-B1.1</i>	<i>PSY1-A1</i> x <i>LPX-B1.1</i>	season	season x <i>PSY1-A1</i>	season x <i>LPX-B1.1</i>
n of lines		53	49	57	47							
2019-2020	b*	18.49-14.83 16.95±0.75	19.58-16.26 17.75±0.68	19.58-14.83 17.24±0.86	19.09-15.32 17.43±0.75		*** 25%	ns	ns	*** 33%	ns	ns
	YPC (mg β-car)	12.05±6.02 9.03±1.38	13.48-6.73 10.56±1.51	13.02-6.02 9.76±1.82	13.48-7.68 9.78±1.37		*** 20%	ns	ns	** 3%	ns	ns
	YPCd (mg β-car)	12.68-5.86 8.65±1.54	13.80-6.73 10.23±1.52	12.05-5.86 8.73±1.60	13.80-6.27 10.23±1.53		*** 21%	*** 17%	ns	*** 14%	ns	ns
	YPL %	26.86-0 6.58±7.62	34.79-0 5.92±7.39	34.79-0 10.55±7.41	18.29-0 1.18±3.27		ns	*** 37%	ns	*** 11%	ns	* 1%
2020-2021	b*	17.57-14.49 15.75±0.77	18.3-15.08 16.63±0.60	17.58-14.39 16.08±0.82	18.3-14.56 16.23±0.86		*** 28%	ns	ns			
	YPC (mg β-car)	10.54-7.12 8.52±0.86	13.09-7.64 10.05±1.03	13.09-7.18 9.18±1.33	11.47-7.12 9.31±1.04		*** 39%	ns	ns			
	YPCd (mg β-car)	10.69-5.17 7.47±1.31	11.92-5.99 8.83±1.44	11.31-5.17 7.34±1.19	11.92-5.50 9.07±1.32		*** 21%	*** 30%	ns			
	YPL %	37.02-0 14.05±11.32	43.78-0 13.03±12.78	42.73-0 19.92±10.24	43.78-0 5.68±9.03		ns	*** 35%	ns			

* p-value <0.05; ** p-value <0.01; *** p-value <0.001; ns: non-significant.

The segregating population genotyping for the *PSY1-A1* and *LPX-B1.1* revealed, as expected, a 1:1 independent segregation for both genes (Table 20). Two advanced lines were heterogeneous for the *PSY1-A1* marker and were not included in the analysis employing *PSY1-A1* as factor. Subsequently, we explored the influence of each gene over the analyzed traits. Our analysis demonstrated that *PSY1-A1* significantly influenced b*, YPC and YPCd in both seasons, but did not affect YPL % (Table 19, Figure 25). *LPX-B1.1* significantly influenced YPCd and YPL % but not b* or YPC (Table 19, Figure 25). No interaction between both genes was significant for any of the traits, including YPCd associated with both genes (Figure 26, Table 19). We also explored the differences among seasons and the interactions between environment and both genes. Environment was associated with all the analyzed traits. However, only a slightly significant interaction was detected between *LPX-B1.1* and the season for YPL % (Table 19).

Table 20: Genotypic characterization Population segregation for each of the polymorphic loci.

n of accessions		<i>LPX-B1.1</i>	
		<i>aa, ac</i>	<i>cc</i>
<i>PSY1-A1</i>	<i>aa</i>	28	25
	<i>al</i>	2	0
	<i>ll</i>	27	22

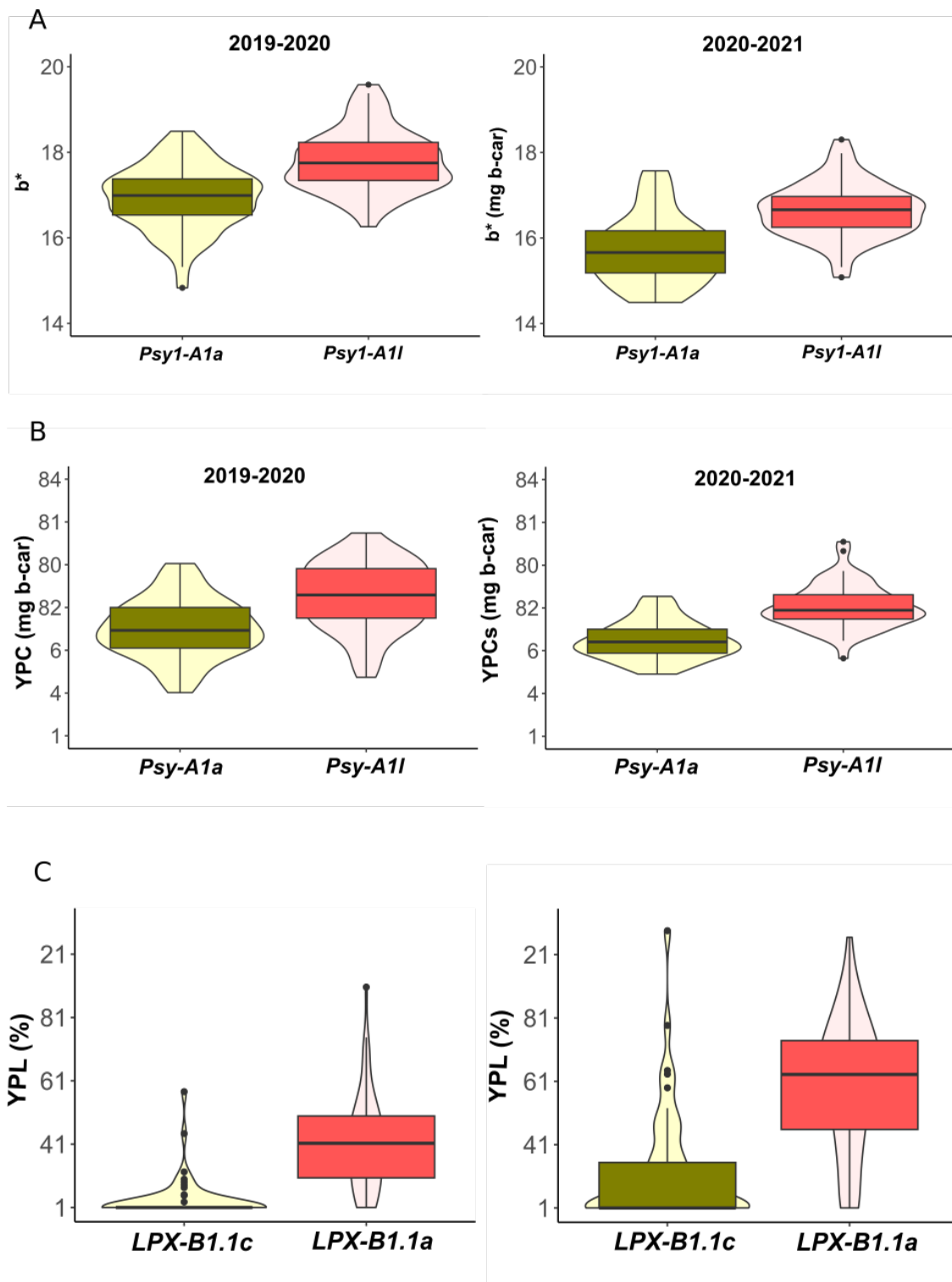


Figure 25: Differences in b^* , YPC, YPL % in each season by *PSY1-A1* or *LPX-B1.1*

Note: Only significant differences are included (p -value <0.05). **A.** b^* value from the colorimeter. **B.** yellow pigment content (YPC). **C.** percentage of yellow pigment loss (YPL%).

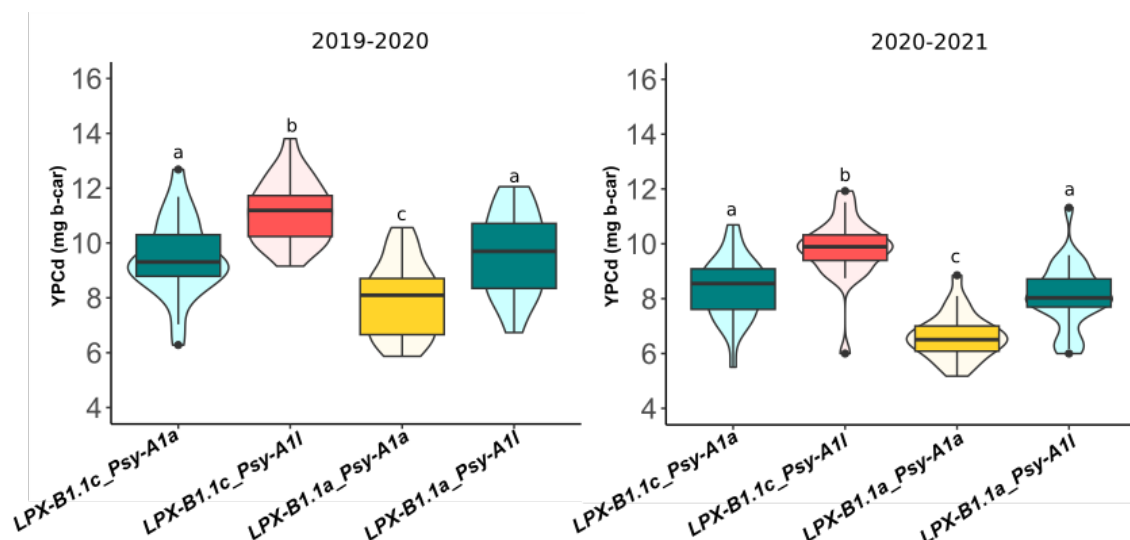


Figure 26: Differences in YPCd in each season by genotype

Note: Colors and letters indicate the significant groups by Tukey test (p-value < 0.05).

4.5.3. Effects of *PSY1-A1* and *LPX-B1.1* in the final YPCd provide clues for breeders

As we have described, *PSY1-A1* genotype influences the b^* , YPC and YPCd. We observed that the individuals carrying the *Psy1-A1l* allele presented higher values for those traits for both seasons (Table 19, Figure 25, Figure 26). Thus, we explored the effect of the *PSY1-A1* on the three traits. Our model indicated that the presence of the allele increased *Psy1-A1l* the yellow flour color (b^*) 0.88 and the yellow pigment content (YPC) 1.52 (mg), with respect to the allele *Psy1-A1a*. The yellow pigment content in dough (YPCd) was also increased 1.51 (mg). The predicted values of YPCd were lower to those of YPC, probably due to the role of the *LPX-B1.1* related with carotenoid catalysis (Table 21).

Polymorphism on carotenoid catalysis enzyme *LPX-B1.1* was associated with final YPCd and YPL %. We observed that the final YPCd was lower on individuals carrying the functional allele *LPX-B1.1a* in both seasons (Figure 26, Table 19). Regarding the yellow pigment loss by oxidation (YPL %), values were zero for almost all the individuals carrying the allele *LPX-B1.1c* (Figure 25C),

while the average was 10.55% and 19.92% for the individuals carrying the functional allele *LPX-B1.1a* (Figure 25C, Table 19). We estimated the effect of the gene on those traits and determined that YPCd was reduced 1.56 (mg) by the functional allele, and yellow pigment loss was increased 11.76 % (Table 21).

YPCd trait is the closer one to the final pasta color after cooking, according to our results both processes carotenoid biosynthesis and catalysis determine its value. We explored the joint influence of *PSY1-A1* and *LPX-B1.1*, our analysis shows that the combination of *LPX-B1.1c: Psy1-A1l* provides the higher values for the trait, *LPX-B1.1a: Psy1-A1a* the lower, while *LPX-B1.1c: Psy1-A1a* and *LPX-B1.1a: Psy1-A1l* are not significantly different (Figure 26). Finally, we estimated the joint effect of both genes and determined that allelic combination *LPX-B1.1c: Psy1-A1l* increases the YPCd by 3.19(mg) with respect to *LPX-B1.1a: Psy1-A1a* (Figure 25, Table 21).

Table 21: Effects of the different alleles and genotypic combinations on the phenotype. The predicted values according to the LME are respect to the genotype used as control (marked in bold) in order to highlight the genetic gain that can be achieved. Traits including b* value from the colorimeter, yellow pigment content (YPC), yellow pigment content in dough (YPCd) and percentage of yellow pigment loss (YPL%). Table includes only the alleles for the genetic factors that were significant for each trait.

Trait	Genotype	Effects	Predicted values
b*	<i>Psy-A1a</i>	-	16.33
	<i>Psy-A1l</i>	0.88	17.21
YPC (mg β -car)	<i>Psy-A1a</i>	-	8.76
	<i>Psy-A1l</i>	1.52	10.28
YPCd (mg β -car)	<i>Psy-A1a</i>	-	8.07
	<i>Psy-A1l</i>	1.51	9.58
	<i>LPX-B1.1c</i>	-	9.64
	<i>LPX-B1.1a</i>	-1.56	8.08
	<i>LPX-B1.1c:Psy1-A1a</i>	-	8.91
	<i>LPX-B1.1c:Psy1-A1l</i>	1.57	10.48
	<i>LPX-B1.1a:Psy1-A1a</i>	-1.62	7.29
	<i>LPX-B1.1a:Psy1-A1l</i>	-0.06	8.85
YPL %	<i>LpxB1.1c</i>	-	3.3
	<i>LpxB1.1a</i>	11.76	15.06

5. Discussion

5.1. Image-assisted quantification of high and low molecular weight glutenin fractions in wheat by SDS-PAGE

Qualitative and quantitative analysis of glutenins may allow the prediction of flour properties, and thus aid selection in wheat breeding programs for development of wheat varieties targeted for higher technological quality (revised in Helguera et al., 2020). However, while qualitative composition is regularly screened and selected in breeding programs, quantification includes usually only total grain protein content, the amounts of different prolamins being not yet regularly analysed. In the present work, we compared the performance of the standard RP-HPLC based quantification protocol (Wieser et al., 1998), with the quantification by electropherograms image analysis obtained following a standard *GLU-3* loci allele profiling protocol (Annex1). For that purpose, and as previous studies to set up similar protocols (Cinco-Moroyoqui & MacRitchie, 2008; Suchy et al., 2007), we analysed a set of genotypes derived from a bi-parental cross.

Even though our segregating population was derived from two lines with identical HMW-GS alleles, it presented a wide range of variation for the rest of parameters analysed (Table 5). For total gliadins we obtained a range between 4.9 and 18.9 much higher than the variability described by Wieser et al. (2023b). Actually, it was even higher than that found by these authors between different species (einkorn, emmer, durum wheat, spelt, and common wheat). Regarding total glutenins, our genotypes presented an average of 2.53 (range 1.4 to 3.9), higher to what was described for common wheat (1.2 to 2.8), but a little lower than when all wheat species were considered (0.5 to 3) (Wieser et al., 2023b). Our population also presented an average GLI/GLU ratio greater than other collections (Suchy et al., 2007; Barak et al., 2013; Dhaka et al., 2015). On this point, it can be noted that while the gliadins to glutenins ratio seems to have significantly decreased during wheat breeding (Wieser et al., 2006; Wieser et al., 2023b).

Our population was obtained by crossing two wheat landraces. However, and as expected considering the lack of segregation for HMW-GS, differences in HMW/LMW ratios were low (0.18 to 0.54), although not so narrow when compared to the reported in other analyses (Dhaka & Khatkar, 2015). The monomorphism for HMW-GS can also explain that no significant correlation was found between

this parameter and SDSs test results, while LMW-GS and total glutenins were positively correlated (Figure 11).

SDS-PAGE has been used for glutenins quantification in previous studies (Dhaka & Khatkar, 2015; Koga et al., 2016) but a standard protocol for this analysis has not yet been proposed. As we wanted to focus on glutenins, we decided to employ the classical extraction protocol of Singh et al., (1991) with small modifications. This procedure, that precisely remove the gliadins, is commonly used for glutenins allelic profiling. Moreover, it was considered the best method for SDS-PAGE-based HMW and LMW-GS quantification after comparison between different extraction protocols (Cinco-Moroyoqui & MacRitchie, 2008). During our analysis we addressed the main inconsistencies found by these authors, such as difficulties in preparing reproducible gels, dependence of band intensity on the content of basic groups of the amino acids lysine, arginine and histidine and reduction of the band intensity during the destaining process. Moreover, we also considered that we were using ethanol (a solvent for some glutenins) when staining the gels.

To unify the SDS-PAGE procedure we have presented a detailed protocol as Annex 1 and highlighted the steps that according to our study have greater impact in reproducibility. Regarding to the staining and destaining steps, it must be noted that HMW-GS and LMW-GS present a similar amino acid composition, thus the procedure will similarly affect the samples inserted in the same gel. Hence, we performed a relative quantification. In order to do so, we included a control sample (always the same genotype from a single extraction) in each gel and could then standardize the obtained values, dividing them by their gel control sample value. Besides, we never destained the gels for more than 16 hours before performing image capture. Moreover, we employed a Molecular Imager® Gel Doc™ XR+ System (Bio-Rad) that has been proved suitable for unknown protein quantification (Alonso Villela et al., 2019).

Nevertheless, any camera with similar resolution, coupled with an image analysis software, that accepts raw image files should also provide reliable results. With this approach we found correlations between SDS-PAGE replicates greater than 0.84 for HMW, LMW and total glutenins. Demonstrating that high repeatability between SDS-PAGE replicates is possible, the main limitation addressed by Cinco-Moroyoqui & MacRitchie (2008) is solved.

As described in the results section, repeatability was a little lower between extraction replicates, thus extraction duplicates should always be performed. We obtained an average coefficient of variation of 6.8, 6 and 5.8 for total, HMW and LMW glutenins respectively. These values are quite close to the results obtained by Wieser et al., (1998) when setting up the standard RP-HPLC procedure (average CV between 2 and 3 for total HMW and LMW glutenins, with a maximum of 5.6). It can be highlighted that estimation of HMW/LMW ratios, where no normalization by the control sample is needed (see the results section), was very accurate, with highly significant correlations among SDS-PAGE replicates and among extraction replicates (Figure 12A and B) and an average coefficient of variation of 2.4.

When we compared our gel- and RP-HPLC-based results, from different extractions but the same flour samples, correlation values were significantly positive for HMW and LMW-GS, although a little lower than between replicates. We also found a similar degree of correlation between SDSs test and prolamin fractions contents by both procedures. Finally, the correlation between SDS-PAGE and RP-HPLC for HMW/LMW ratio was 0.85, demonstrating the accuracy of our gel imaging based protocol. For this parameter, we detected a significantly negative correlation with SDSs test. This result was apparently surprising since higher HMW/LMW ratios are considered to favour the formation of GMP, thus increasing gluten strength (Wieser et al., 2023b). However, in our case, the lack of segregation of HMW-GS coding loci translates into small differences among genotypes regarding HMW abundance.

Greater ratios are then not due to higher content in HMW-GS but to lower content in LMW-GS, possibly caused by the segregation of the Jeja Pardilla null alleles at *GLU-A3* and *GLU-D3* loci, associated with lower gluten strength (Ikeda et al., 2014). Therefore, lines carrying the Richela Blanca alleles are expected to have both increased LMW content and SDSs volume values. It could be added that all lines in our population carry the *Glu-D1a* allele associated with poor technological properties (revised by Cornish et al., 2006). It may make more remarkable the influence on gluten strength of their genotype differences at LMW-GS loci. Nevertheless, specific relations between prolamins alleles, prolamins quantification and gluten strength are out of the scope of this work, and should be validated under different environments before being thoroughly discussed.

To sum up, here we present a protocol, affordable for breeders and researchers with some experience in glutenins allele profiling. Our protocol, allows to methodologically unify the analysis of two of the three factors that influence GMP

formation: HMW allelic variation and HMW to LMW-GS ratio. It may potentially overperform the actual flour end-use quality predictions based on glutenins alleles and total protein content (revised by Cornish et al., 2006; Helguera et al., 2020). The ranges of prolamin compositional parameters covered by the germplasm subset used suggest that the results found are highly extrapolated. In order to guarantee that our protocol can be set up in different labs, Annex 1 provides a detailed description, marking the specific key steps that need to be considered for quantification.

5.2. Impact of quantitative and qualitative Glutenin Variation on wheat quality within a similar genetic background

The analyses of the association between allelic variation and quantity of glutenins with gluten properties provide valuable information for selecting and improving the end-use quality of wheat. In this study, both qualitative and quantitative effects on gluten quality- measured as GPC and gluten strength (estimated by SDSs)- were investigated by comparing intra-variety biotypes. All genotypes were cultivated under identical environmental conditions, and phenotypic values were compared within a similar genetic background to minimize environmental and genetic variability affecting glutenin contents (Wieser et al., 2023b).

The results revealed that both the quantity and composition of glutenins affected gluten quality, and both aspects were closely interrelated. The glutenin subunit genotype was the primary determinant of variation for all parameters examined, except LMW-GS amount. These findings confirmed that the biotypes inside a variety may display differences in key traits for wheat breeding (Metakovsky et al., 2024). The non-significant effect of genotype on LMW-GS content could be attributed to the small differences in this fraction among the studied genotypes, as previous studies determined that glutenin contents were predominantly genotype-dependent (Panozzo & Eagles, 2000; Zhang et al., 2007). The strong influence of the genotype on SDSs, accounting for 90% of the observed variation, is likely due to the study's focus on evaluating phenotypic effects in biotypes with largely similar genetic backgrounds.

Regarding the glutenin-encoding loci, it is noteworthy that most of the *GLU-1* alleles analysed in this study are among the most common in Spanish landraces (*Glu-A1b* and *y*, *Glu-B1e*, *Glu-D1a*, *c* and *d*) (Lopez-Fernandez et al., 2021).

Consequently, the results obtained may hold particular relevance for utilizing this germplasm in Spanish pre-breeding programs aimed at improving end-use quality.

Although our study could not specifically analyse the relationship between certain subunits and quality, previous research on Spanish landraces reported that the *Glu-A1y* allele (subunit 1Ax2..) was associated with superior SDSs values compared to *Glu-A1b* (subunit 1Ax2*). Additionally, both the *e** and *d** alleles (subunits 1Bx20+1By20 and 1Bx6+1By8, respectively) exhibited low SDSs values without significant differences (Lopez-Fernandez et al., 2021). The *Glu-D1d* is generally associated with stronger gluten than *Glu-D1a*, but the pronounced effect of the *Glu-D1d* allele may, in some cases, mask the positive contributions of less influential alleles.

The *GLU-D1* locus, which controls HMW-GS, was the primary determinant of quality, HMW-GS content and HMW/LMW ratio across genotypes (Table 8). In this study, the effects of *GLU-D1* alleles on the analysed parameters were attributable to variation in the x-type subunit, as all genotypes shared the 1Dy12 y-type subunit. Previous studies have shown that x-type HMW-GS play a much greater role in wheat quality compared to LMW-GS and y-type HMW-GS (Wieser & Kieffer, 2001). This could be due to the x-type HMW-GS having a higher abundance and distinctive molecular characteristics, such as disulfide structures, which promote the development of a stronger gluten network (Gianibelli et al., 2001). In contrast to the high influence of *GLU-D1* on quality, *GLU-A1* contributed modestly to the variation in the analysed parameters. Zhou et al. (2021) demonstrated that *GLU-D1* overlaps with a QTL cluster that regulates HMW-GS content, consistent with the effects of *GLU-D1* on HMW-GS amount observed in our study.

For the *GLU-3* loci, *GLU-A3* explained the largest proportion of variation across all parameters analysed (except for LMW-GS content), while *GLU-B3* and *GLU-D3* were found to be negligible. The influence of *GLU-A3* on gluten strength aligns with earlier findings, such as those by Payne et al. (1987). Furthermore, specific genes on chromosome 1AS have been identified to control HMW and LMW-GS amounts, whereas no QTLs for LMW-GS have been identified on chromosome 1BS (Zhou et al., 2021).

In this study, none of the glutenin loci had a significant effect on LMW-GS content, consistent with the previously noted low influence of genotype on this parameter. Notably, most of the alleles identified at *GLU-A3* have not been

previously described, a finding that is common among wheat landraces (Lopez-Fernandez et al., 2021).

Comparisons of the phenotypic values of the most frequent biotypes enabled accurate estimation of the effects of different glutenin alleles within the same genetic background. Mean comparisons between genotypes differing only in *GLU-D1* alleles (gt 1, gt 2, and gt 3 in Table 7) confirmed the superiority of *Glu-D1a* over *Glu-D1c* for SDSs, consistent with findings by Payne & Lawrence (1983). Low for SDSs and GPC values were also observed in the variety carrying *Glu-D1c* ('Marius'). Additionally, *Glu-D1a* exhibited higher HMW-GS content and HMW/LMW ratio compared to alleles *l* and *c*, and higher GPC than allele *c*. The *Glu-D1l*, which codes only for 1Dy12 (lacking 1Dx2), showed the highest GPC but the lowest glutenin fraction contents. Recent studies have highlighted the lowest values of null alleles at *GLU-D1*, or triple null at *GLU-1*, for gluten strength, glutenin contents and HMW/LMW ratio. These triple null lines also exhibited higher GPC values (Liu et al., 2022; Dai et al., 2024). The absence of 1Dx2 has been reported to impair dough quality by delaying glutenin polymerization (Song et al., 2020), which may be advantageous for improving biscuit-making flours (Dai et al., 2024).

The association between protein fractions and quality was more evident when genotypes were analysed separately based on their *GLU-D1* alleles. This result demonstrated that the relationships between glutenin contents and quality were highly dependent on glutenin allelic variation. These findings raise the question of whether quantitative differences in glutenins among alleles may contribute to their differential effects on gluten quality. A strong positive relation between SDSs and HMW-GS content, and HMW/LMW ratio in gt 1 and gt 2 (Table 10) confirmed that the inferior gluten strength of allele *Glu-D1l* (gt 1) compared to *Glu-D1a* (gt 2) was associated with the lower HMW-GS content and HMW/LMW ratio of *Glu-D1l* (Table 10). Conversely, GPC was the primary factor influencing gluten strength when analysing *Glu-D1a* and *c** alleles (gt 2 and gt 3 in Table 10), although HMW-GS content and HMW/LMW ratio also affected the quality parameters. Based on these findings, the inferior gluten strength of *Glu-D1c* and *Glu-D1l* compared to *Glu-D1a* was attributable to different causes; the lower HMW-GS content and HMW/LMW ratio of *Glu-D1l*, and the lower GPC of *Glu-D1c*. Thus, the superior gluten strength of *Glu-D1a* appears to result primarily from its higher HMW-GS content and HMW/LMW ratio, alongside adequate GPC levels.

In the lines differing only in their *GLU-3* composition, distinct relationships between quality parameters and glutenin amounts were detected between genotypes with the *Glu-D1c* allele (gt 3, gt 7, and gt 9 in Table 10) and those with the *Glu-D1a* (gt 2 and gt 4 in Table 10). Specifically, GPC had a greater influence on gluten strength when *Glu-D1a* was present, while the effects of HMW-GS amount and HMW/LMW ratio were more pronounced in genotypes with *Glu-D1c*. Interestingly, LMW-GS content significantly affected GPC only in genotypes carrying the *Glu-D1a* allele. This positive relationship between LMW-GS and GPC may be attributed to the lower LMW-GS and GPC values observed in gt 4, as well as the positive association between both parameters in the lines of gt 2 ($R^2 = 0.31$). Overall, the relation between HMW-GS amount and GPC strengthened within genotypes sharing the same *GLU-D1* allele ($R^2 > 0.40$, Table 10). Similar findings were reported by Dhaka & Khatkar (2015), who noted that gliadin and glutenin contents positively affected dough strength in lines with the same *GLU-D1* allele. According to these results, the outstanding SDSs values of gt 9, despite carrying the less favourable *Glu-D1c* allele, were likely driven by its higher GPC, HMW-GS content and HMW/LMW ratio. In contrast, the unexpectedly lower SDSs value of gt 4 compared to gt 2 appeared to result from its lower HMW-GS content and, particularly, GPC. Both gt 4 and gt 9 differed from the other genotypes with the same *GLU-D1* alleles in their LMW-GS encoded by *GLU-3*. These results suggested that allelic variation at *GLU-3* loci can differentially affect the quality and quantity of glutenins associated with the *GLU-D1* alleles. This aligns with observed inconsistencies when comparing *GLU-D1* means across all genotypes, irrespective of their *GLU-A1*, *GLU-B1* and *GLU-3* compositions (Table 9). The phenotypic values of varieties confirmed that *GLU-D1* alleles alone do not always provide the best prediction of gluten strength (e.g. ‘Berdun’ vs. ‘Califa Sur’), emphasizing the importance of considering allelic variation at other glutenin genes.

Overall, GPC was the parameter most strongly influencing gluten strength. Some studies reported a positive relation between GPC and dough strength (e.g. Wieser & Kieffer, 2001). A sufficient amount of gluten proteins is necessary for developing a continuous proteinaceous network in the dough, as well as for stabilizing gas bubbles in leavened doughs and pore walls in baked goods (Wieser et al., 2020b). However, not all studies have found a relation between both quality parameters (see Weegels et al., 1996 for a review). It has been suggested that this

relationship weakens when the protein content range of the samples is narrow (Dhaka & Khatkar, 2015).

In this study, HMW/LMW ratio displayed a positive effect on both quality parameters, except in genotypes carrying the *Glu-D1a* allele or *GLU-D3* alleles (*b* or *c*). Previous studies have reported conflicting findings regarding the relationships between HMW/LMW ratio and gluten strength. While Uthayakumaran et al. (2000) found a positive association, others found no significant relationship (Wieser & Kieffer, 2001; Zhang et al., 2007).

Our study also showed the importance of HMW-GS amount for quality parameters, although its influence on gluten strength seemed to be due to its positive relation with GPC. This relationship between GPC and total gluten protein content has been similarly reported by Koga et al. (2016). Numerous studies have corroborated the positive impact of HMW-GS content on quality (Singh et al., 1990; Hoseney, 1994; Johansson et al., 2001; Wieser & Kieffer, 2001; Dhaka & Khatkar, 2015; Wieser et al., 2023b). This glutenin fraction contributes to forming the backbone of glutenin network, thereby increasing the content of glutenin macropolymers and enhancing gluten strength (Wieser et al., 2023b; Ribeiro et al., 2015; Dhaka & Khatkar, 2015). In agreement with other studies (Hoseney, 1994; Dhaka & Khatkar, 2015; Johansson et al., 2001), our findings demonstrated that gluten strength was influenced by both the amount and type of HMW glutenin subunits. Subunits encoded at *GLU-D1* and *GLU-B1* were particularly significant.

Our results showed that LMW-GS content was generally irrelevant to quality parameters, except for its positive relationship with GPC in genotypes with the *Glu-D1a* allele. Other study similarly reported a positive relation between LMW-GS content and GPC (Zhang et al., 2007). However, in contrast to our results, other studies have identified positive associations between LMW-GS amount and gluten strength (Wieser & Kieffer, 2001; Zhang et al., 2007; Suchy et al., 2007). It has been shown that HMW-GS contribute more positively in quantity to dough strength than LMW-GS, as approximately twice the quantity of LMW-GS is required to achieve a comparable effect (Uthayakumaran et al., 2000; Wieser & Kieffer, 2001). As we noted earlier, the small differences in LMW-GS content among the genotypes evaluated in this study likely explain the lack of significant effects of LMW-GS on quality parameters in our results.

To sum up, biotypes within the same variety offer a robust framework for accurately identifying genes and molecular markers associated with quality-relevant parameters. This study highlights the importance of considering glutenin subunit composition when evaluating the effects of glutenin fractions on quality traits.

5.3.A new genetic map in durum wheat, allows the detection of QTL related to yield and grain protein content

Several studies have been developed in order to uncover the genetic determinants of yield and quality-related traits (Colasuonno et al., 2021; Soriano et al., 2017). Lately, GWAS have been favoured over traditional linkage mapping, as experimental populations development requires several years, even with the speed breeding protocols now available (Watson et al., 2018), and the construction of high-density genetic. However, we must not forget that experimental populations, developed from controlled crosses between polymorphic parents, are one of the best resources available to deepen the genetic control of complex traits and identify the genomic regions responsible for them (Xu et al., 2017). In the case of wheat, experimental populations have allowed and continue to facilitate the identification of a large number of important QTLs (quantitative trait loci) for the improvement of the species. These include regions responsible for resistance to pathogens (Desiderio et al., 2021), yield (Arraigada et al., 2020), root morphology (Soriano & Alvaro, 2019), heading date (Guedira et al., 2016), and quality (Guo et al., 2020). Moreover, once a QTL is found, experimental populations allow the quick development of recombinants inside the QTL region and the positional cloning of the responsible genes. In this work we have focused on the Endural x Aldura population developed from two different durum wheat cultivars released in the 1980s. We characterized the population for five yield-related traits (grain area, perimeter, major and minor ellipse, plus TKW), grain protein content, and flour color. The population presented a wide range of variation for the traits analysed (Table 11).

For yellow flour color (b^*) lines had a range of almost 5 points in season 2019-2020 (from 14.83 to 19.58), a third of what was found in a collection of more than one hundred Mediterranean durum wheat cultivars (10.32 to 25.78) (Parada et al., 2020). Actually, Parada et al. (2020) selection included landraces and modern cultivars providing a high diversity. This trait had a positive correlation among all the years analysed except 2012-2013 and 2019-2020. However, for 2012-2013 only

grains of 79 genotypes were available, and those grains were 10 years old when the analysis was performed. When we compared the results for 2019-2020 and 2020-2021, the correlation was 0.45 (Figure 17), suggesting an important influence of genotype on the trait. For grain protein content values ranged almost a 7% in 2019-2020 (from 10.98% to 17.27%) (Table 11), covering almost the range described for ancient durum wheats (from 12% to 18%) reviewed by (Cabas- Lühman et al., 2020). The correlations being lower than for yellow color (maximum of 0.4, Figure 17) and nonsignificant in two comparisons. This is expected, as the protein content is a quantitative trait with low heritability, largely influenced by the environment (Blanco et al., 2012). Regarding grain traits, if we focus on TKW, the population presented differences of 25g in 2020-2021 (from 32.53 to 57.67), a wider range that described for ancient durum wheats (38.8-49.3 reviewed by (Cabas- Lühman et al., 2020). For this trait correlations among years reached 0.47, however, were nonsignificant between 2012-2013 and 2013-2014, 2019-2020 (Figure 17), probably due to the lower number of lines as stated before for yellow color. The phenotypic results highlight the diversity of the Endural x Aldura population and its suitability for QTL mapping.

The population was genotyped with DArT-seq technology to construct a high-density genetic map. After filtering out low quality and no-polymorphic markers, the obtained genetic map contained 2117 loci, including more than one thousand markers not located in the durum wheat reference genome (Maccaferri et al. 2019). The final map, comprised 17 linkage groups, assigned to the 14 durum wheat chromosomes, with chromosomes 3B, 5A and 5B including two linkage groups, probably due to the lack of polymorphic markers in those regions (Figure 18). The lack of linkage between markers belonging to the same chromosome is common in durum wheat mapping and was also an issue for the construction of several durum wheat consensus maps. Marone et al. 2012 consensus map included 1898 loci arranged in 27 linkage groups, and Maccaferri et al. (2014) consensus map included 2575 markers in 17 linkage groups (two linkage groups for chromosomes 1A, 2A and 3A). The Endural x Aldura map contained 2117 markers, close to the average for maps developed since 2015. That ranged between a maximum of 10911 markers to 395 for Svevo x Zavitan to Kunduru-1149 x Cham-1 respectively reviewed by (Colasuonno et al. 2021). The Endural x Aldura map spanned 1471.1 cM, being slightly smaller than the last available maps (1436 (Rusty x PI192051-1) to 4854cM (Kunduru-1149 x Cham 1). The population has an average of 1.44 markers/cM, which is suitable for QTL mapping.

Based on the markers located in the durum wheat reference genome (Maccaferri et al. 2019), we compared the genetic map with the reference genome (Annex 2). Chromosomes 2B, 3A, 4B, 6A, 6B, and 7A presented inversions, suggesting good anchoring but inverse sense when anchoring scaffold to construct the reference genome. Chromosomes 3B and 4A presented several rearrangements. Besides, chromosomes 1A and 2A covered a smaller portion of the reference genome, as expected considering they included the lowest number of markers (53 and 30 respectively). The new version of the durum wheat genome now in progress (Peter Haugurd et al., 2024) should enhance the anchoring and resolve some of the detected issues, as have been done in bread wheat (Zhu et al., 2021).

Finally, QTL analysis revealed associations, in most of the durum wheat chromosomes, except 1A, 2A, 6A and 5A (Annex 3). It must be noted that 1A, 2A and 5A were the chromosomes that included a lower number of markers (Table 12), while 6A was the second most covered one. QTLs were found for all the traits, however TKW did not present stable associations (Table 13). QTLs detected in several environments (stable QTLs) offer more reliable targets for selection. Our analysis revealed two stable QTL associated with quality-traits. One in chromosome 1B (54-70 cM), associated to grain protein content. This location included the *GLU-B1* locus, polymorphic in the Endural X Aldura population (see results section 4.4), said to affect not only gluten strength but also protein content in some environments (Roncanello et al., 2021). And another in chromosome 7A (86-117cM) associated with yellow color (b*). This QTL explained around a 30% of the yellow color variation in the seasons 2019-2020 and 2020-2021, around 15% for 2013-2014. This QTL overlaps with the position of *PSY-1A** gene, known to be the key step in carotenoid biosynthesis and one of the genes underlying the differences in flour color (He et al., 2008; Colasuonno et al., 2019).

Regarding grain and yield traits, our analysis identified five stable QTL. Two of them on chromosomes 3B and 6B associated with only one trait (grain major ellipse and grain perimeter, respectively). The QTL in 3B (80-81Mb) overlaps with a previously identified QTL associated with grain ASPECT (Mangini et al 2025), for which a MADS-box transcription factor (*TRITD3BvG278870*, 82.58Mb) is considered a strong candidate. The QTL in 6B is only 10 Mb away from a previously identified one (Mangini et al 2021) associated with TKW and grain length. The other QTLs were located on homeologs group 4, and harbour the higher number of associations (Figure 20). The chromosome 4B region includes associations to all

the grain size and shape traits, plus TKW in 2013-2014. This region showed an inversion with respect to the reference genome. However, we could determine that it includes the gene *RHT-B1* (29Mb), known to co-localize with several yield related QTLs (Taranto et al 2023a). The chromosome 4A included two stable QTLs at the end of the chromosome (more than 600Mb) for which not clear co-localization with previous QTLs or candidate genes were found.

The characterization of Endural x Aldura population revealed a wide variability and the suitability of the population for QTL mapping. We have been able to accurately find an association between yellow color and the already known *PSY1-1A* gene. Thus, we present a polymorphic biparental population that might aid in the positional cloning of the 3B QTL previously detected, and the rest of stable QTLs identified.

5.4. Identification of genetic factors controlling rheological properties in durum wheat by QTL detection

Most of the studies exploring durum wheat end-use quality in a wide range of genotypes focus mainly on flour color, GPC and SDSs (Subira et al., 2014; Giraldo et al., 2016; reviewed by (Colasuonno et al., 2021) due to the simplicity of colorimeter, NIR and SDSs methods. However, it is well known that quantity of specific prolamins greatly influence durum wheat quality. Moreover, starch (damage starch) and arabinoxylans also play a role. In fact, high starch content changes the functional properties of the flour, such as the gelatinization, pasta characteristics, and baking applications (Seib, 1994). Until the last decade the lack of cheap micro methods that could be easily applied to large sample collection has discouraged more specific analysis. In this work we take advantage of the last micro methods developed, SRC (Guzman et al., 2015) and prolamins quantification based on SDS-PAGE (Bouabdellah et al., 2024, described previously in section 4.1). We use those methods to extend the characterization of the Endural x Aldura population. Those materials, developed from Endural, a variety which exhibits very good rheological properties, and Aldura, characterized by its inability to form gluten despite having a prolamins composition similar to that of other wheats (Dr. Patricia Giraldo personal communication) provide an ideal frame to detect genomic regions governing quality traits in durum wheat. The analysis demonstrated that the population presented transgressive lines for all the traits studied (quantification of HMW-GS, LMW-GS and estimation of HMW-GS to

LMW-GS ratio; gluten strength estimation; and percentage of solvent retention capacity for five different solvents Table 14). As example gluten strength estimation (SDSs test) results ranged from 18 to 45mm, while both parent lines were 21.5mm on season 2020-2021. When we analyzed correlations between seasons we found positive significant correlations among all the traits, suggesting an important genotypic effect.

As it is well known prolamins, specially LMW-GS are the major determinants of durum wheat quality (Ruiz & Giraldo, 2021). In our population the parental lines were polymorphic for several prolamin loci (Table 4). Thus, we genotyped the population and analyzed the individual effect of each locus on the nine characterized traits. HMW-GS to LMW-GS ratio was not associated with any prolamin loci, while HMW-GS and LMW-GS quantity were associated at least in one season with all the polymorphic glutenins. Suggesting that other loci might be governing the exact glutenin balance. Zhouh et al. (2021) identified QTLs in 16 of the 21 bread wheat chromosomes associated to total glutenin and its fractions, with an important QTL cluster in chromosome 6A. Besides, Storlie et al. (2009) also highlighted the effect of different genomic regions on HMW-GS loci expression.

For SRC, sodium carbonate (S4) related with damage starch was not associated to prolamin loci as expected. While the rest of SRC test showed associations, even though sucrose (S5) is supposed to be related with arabinoxylans contents (Guzman et al., 2015). All the solvents and the SDSs test presented the same tendencies when associated with prolamin loci. For HMW-GS, advanced lines carrying *Glu-B1d* (6+8, from Aldura) presented higher SRC than those carrying *Glu-B1e* (20x+20y, from Endural), in accordance with previous studies (Roncanello et al., 2021; Ruiz & Giraldo, 2021). For the LMW-GS Endural carries the alleles (*Glu-A3l*(6+10), *Glu-B3w*(2+4+15+17), *Glu-B2a*(12)), and Aldura (*Glu-A3k*(5), *Glu-B3s*(8+9+13+16), *Glu-B2b*(null)). *Glu-A3l* had positive effect on SDSs and SRC, in accordance with previous studies (Carrillo et al., 2000; Roncanello et al., 2021; Ruiz & Giraldo, 2021). For the other two polymorphic loci studies are controversial, with even some that found no association between allelic variability and rheological properties reviewed by (Ruiz & Giraldo, 2021). In our analysis, *Glu-B2b* had a clear negative impact on SRC and SDSs in all the traits. Although, it must be noted, that it was not significant for water and alkaline water retention capacity in 2019-2020. At *GLU-B3* locus, results were highly significant (Table 16), with *Glu-B3w* (2+4+15+17) presenting a positive effect, even though the population was also

segregating for other loci. The Endural x Aldura population, could be used to clarify the interactions between HMW-GS and LMW-GS in gluten strength. However, such analysis is out of the main scope of this thesis, identifying new genomic regions, and will be explored in future analysis.

We took advantage of the high-density genetic map previously described (section 4.3) and identified the genomic regions associated with end-use quality traits. We detected associations for all the traits, in all chromosomes except (1A, 2A, 2B and 5A). It must be noted that 1A, 2A and 5A were neither associated with yield related traits, GPC or flour color, and were the ones with the lower number of polymorphic markers (described in section 4.3). Thus, this lack of associations simply reflects a lack of polymorphism in the analyzed population.

Most of the associations were only identified in one of the seasons, highlighting the important effect of the environment on end-use quality, as previously described (Zingale et al 2023). Stable QTLs were detected at four different locations on chromosome 1B and 6B (Figure 23). On chromosome 1B one (31-73.5cM, 383-590Mb) overlapped with the *GLU-B1* locus (548Mb) and with a stable QTL associated with GPC (section 4.3). While the other located at the beginning of the chromosome 1B (0-18cM) will probably overlap with LMW-GS coding loci (19Mb). The associations at chromosome 6B were for SDSs test and did not overlap but were close to a previously identified stable QTL for grain perimeter (section 4.3). Besides, the polymorphic *GLI-B2* locus was not associated with SDSs test (Table 16), nor could we identify any potential candidate gene. Colasuanno et al., (2021) and Kiszonas & Morris (2017) reviews about durum and bread quality QTLs, did not include any association with gluten strength inside 6B.

Our analysis highlighted the important role of the environment on durum wheat quality. Moreover, it highlighted the role of chromosome 6B on gluten strength, in which positional cloning could be the best strategy in order to identify the gene governing gluten strength.

5.5. Investigating *Phytoene Synthase 1* and *Lipoxygenase 1* Effects on Wheat Flour Color: Clues for Breeding High-Quality Durum Wheat

As durum wheat is mainly employed for human consumption, the end-use quality must be considered in breeding programs (Zingale et al., 2023). During the last century, breeding has produced an increase of several quality related traits,

like, yellow color index (b^*). For this trait cultivars released between 1988-2000 reached average values of 14.5, around 10% more than cultivars released before 1945 (Subira et al., 2014). Our work revealed that there is room for improvement. Endural (released in 1989) presented a value of 14.54 (season 2020-2021), while Aldura (released in 1986) had higher values (17.98 season 2020-2021).

Determining the best predictor for the final trait value is key to breed for pasta color, a trait difficult to screen in segregating generations. Troccoli et al. (2025) analysis suggested that yellow index in flour is the best predictor for pasta color (correlation 0.93). Our analysis, however, revealed a correlation between b^* and YPCd of 0.49 and 0.3 (in 2019-2020 and 2020-2021, Figure 24). This is more in accordance with Borrelli et al. (1999), which indicated that lipoxygenase activity affects pasta color. Besides, Ficco et al. 2014 indicated that percentage of carotenoid loss for pasta produced from semolina ranges from 4% (high YI and low LOX activity) to 20% (low YI and high LOX activity), suggesting a possible interaction between both traits. That was not shown by our analysis, Endural presented lower YPC and lower YPL % (Table 18). Moreover, we do not detect any significant correlation between YPC and YPL % (Figure 24), indicating that both processes should be considered in breeding programs.

Determining the final pasta color in segregating populations is not feasible, considering the number of genotypes managed during the first stages of any breeding program. Marker assisted selection allows to overcome those difficulties, if alleles effects and gene interactions are known reviewed by (Colasuonno et al., 2019). We analyzed the polymorphism present in our segregating population for four carotenoid synthesis (*PSY1-A1*, *PSY1-B1*, *PDSB*, *ZDSA*) and two carotenoid catalysis genes (*LPX-B1.1* and *LPX-A3*), detecting only polymorphism in *PSY1-A1* and *LPX-B1.1*. Contrary to previous studies (Parada et al. 2020) in our population only a slight interaction between *LPX-B1.1* and the environment was detected for YPL % (Table 19, Figure 25C). Both results confirm that our population provides an ideal resource to estimate the effects of each individual gene.

Previous works (Sing et al., 2009; He et al., 2009; Campos et al., 2016; Parada et al., 2020), explored the role of the different *PSY1-A1* alleles in diverse populations with controversial results. This was probably due to the different genetic backgrounds, genotype by environment interactions, or the possible presence of a transposon as suggested by He et al. (2009). Our analysis demonstrates that *Psy1-A1l* significantly increases the b^* , YPC, YPCd with respect to *Psy1-A1a* (Figure 25, Figure 26), contrary to what was stated by He et al. (2009).

Regarding to *LPX-B1.1*, our results showed that the less functional allele *LPX-B1.1c* (Carrera et al. 2007) produced an increase of 1.56 mg in YPCd due to lower YPL % (Table 21, Figure 26) in accordance with Fu et al. 2013.

Parada et al. (2020), explored the joint role of *PSY-1* and *LPX-1* on durum wheat color. However, they only characterized the semolina color, and only two genotypes carrying the low lipoxygenase allele (*LPX-B1.1c*) were identified among the 119 diverse genotype analyzed. In our study, we focused on YPCd, known to present higher correlation with final pasta color ($r^2 = 0.59-0.90$), than pigment content in semolina according to Fu et al. 2013. Besides, we compared four different genotypic combinations *LPX-B1.1c: Psy1-A11* (low LPX activity & high YPC), *LPX-B1.1c: Psy1-A1a* (low LPX activity & low YPC), *LPX-B1.1a: Psy1-A11* (high LPX activity & high YPC), *LPX-B1.1a: Psy1-A1a* (high LPX activity & low YPC) with shared genetic background (Table 20). Our results clearly demonstrate that *LPX-B1.1c: Psy1-A1a* and *LPX-B1.1a: Psy1-A11* YPCd values were not significantly different, and the best genotype was *LPX-B1.1c: Psy1-A11* presenting an increase of 3.19 (mg) in YPCd with respect to *LPX-B1.1a: Psy1-A1a* (Figure 26)

In summary, a successful MAS strategy for durum wheat color, requires the genotypic characterization for both carotenoid biosynthesis and catalysis genes, followed by the combined selection of lower LPX activity alleles, and alleles associated with higher semolina yellow index.

6. Conclusions

1. The estimation of HMW-GS to LMW-GS ratio based on the SDS-PAGE protocol presented a correlation of 0.85 when compared to the standard RP-HPLC method. Those results demonstrate that, the new protocol allows to methodologically unify the analysis of two of the three factors that influence gluten macropolymer formation: HMW-GS allelic variation and HMW-GS to LMW-GS ratio.
2. The analysis of intra-variety biotypes confirmed that the *GLU-D1* locus was the primary determinant of quality, HMW-GS amount and HMW-GS to LMW-GS ratio. Besides, within genotypes carrying the same *GLU-D1* allele, the relation between HMW-GS content and GPC was strengthened, with GPC being the parameter influencing most gluten strength.
3. To improve gluten strength in genotypes with the same LMW-GS composition, allelic variation at the *GLU-D1* locus and the quantitative composition of glutenin fractions must be considered. Selection strategies should prioritize: increasing GPC, HMW-GS and LMW-GS levels in genotypes with *Glu-D1a*; enhancing GPC, HMW-GS levels and HMW-GS to LMW-GS ratio in genotypes with *Glu-D1c*; and boosting HMW-GS content and HMW-GS to LMW-GS ratio for genotypes with *Glu-D1L*.
4. The durum wheat segregating population Endural x Aldura phenotyping showed the existence of transgressive phenotypes for most of the traits. That variability coupled with the positive correlations found between environments highlight its suitability for QTL mapping.
5. The high-throughput genotyping of the Endural x Aldura population allowed the construction of a high-density genetic map. The map comprised 17 linkage groups, assigned to the 14 durum wheat chromosomes and spanned 1471.1 cM, with an average of 1.44 markers/cM, which is suitable for QTL mapping.
6. QTL analysis for yellow flour color identified a major QTL on chromosome 7A, that overlapped with the previously cloned *PSY1-A1*, the main gene controlling carotenoids synthesis.

7. QTL analysis for TKW, grain size and shape traits identified three major QTL clusters at chromosomes 4A and 4B. The QTL at 4B overlapped with the *RHT-B1* gene, known to be associated with grain yield, while no clear candidates were identified for chromosome 4A QTLs.
8. QTL analysis for grain protein content, solvent retention capacity, glutenin quantification and SDSs test identified a two major QTL clusters on chromosome 1B, one overlapping with the *GLU-B1* coding gene. For this gene the *Glu-B1d* allele increased gluten strength.
9. Two stable associations were found between SDSs and two locations on chromosome 6B. The *GLI-B2* locus polymorphic in the studied population was not associated with SDSs, and no other clear candidate gene was identified.
10. The Endural x Aldura population was polymorphic for one carotenoid synthesis (*PSY1-A1*) and one catalysis (*LPX-B1.1*) gene. *Psy1-A1l* allele significantly increased the yellow flour color, yellow pigment content in flour and yellow pigment content in dough with respect to *Psy1-A1a*. The allele *LPX-B1.1c* produced an increase in yellow pigment in dough due to lower yellow pigment loss by oxidation.
11. To improve yellow pigment content in dough both carotenoid synthesis and catalysis genes must be considered, the best genotypic combination being *LPX-B1.1c: Psy1-A1l*.

References

- Abdel-Aal, E. S. M., Young, J. C., Rabalski, I., Hucl, P., & Fregeau-Reid, J. (2007). Identification and quantification of seed carotenoids in selected wheat species. *Journal of Agricultural and Food Chemistry*, 55(3), 787–794. <https://doi.org/10.1021/jf062764p>
- Aguiriano, E., Ruiz, M., Fité, R., & Carrillo, J. M. (2009). Effects of N fertilisation, year and prolamin alleles on gluten quality in durum wheat (*Triticum turgidum* L. ssp. *turgidum*) landraces from Spain. In INIA) Spanish Journal of Agricultural Research (Vol. 7, Issue 2). www.inia.es/sjar
- Alonso Villela, S. M., Kraïem, H., Bouhaouala-Zahar, B., Bideaux, C., Aceves Lara, C. A., & Fillaudeau, L. (2020). A protocol for recombinant protein quantification by densitometry. *MicrobiologyOpen*, 9(6), 1175–1182. <https://doi.org/10.1002/mbo3.1027>
- Andrade, F., Alvarez, J. B., & Guzmán, C. (2024). Development of a germplasm master set covering variability of high molecular weight glutenin subunits for the GLU-A1 locus in *Triticum* sp. *Journal of Cereal Science*, 118. <https://doi.org/10.1016/j.jcs.2024.103968>
- Arriagada, O. ;, & et al. (2020). Molecular Mapping and Genomics of Grain Yield in Durum Wheat: A Review. *International Journal of Molecular Sciences*, 21(19), 7021. <https://doi.org/10.3390/ijms21197021>
- Arriagada, O., Gadaleta, A., Marcotuli, I., Maccaferri, M., Campana, M., Reveco, S., Alfaro, C., Matus, I., & Schwember, A. R. (2022). A comprehensive meta-QTL analysis for yield-related traits of durum wheat (*Triticum turgidum* L. var. *durum*) grown under different water regimes. *Frontiers in Plant Science*, 13. <https://doi.org/10.3389/fpls.2022.984269>
- Atienza, S. G., Ballesteros, J., Martín, A., & Hornero-Méndez, D. (2007). Genetic variability of carotenoid concentration and degree of esterification among tritordeum (*xTritordeum* Ascherson et Graebner) and durum wheat accessions. *Journal of Agricultural and Food Chemistry*, 55(10), 4244–4251. <https://doi.org/10.1021/jf070342p>
- Autran, J. C. (1994). Size-exclusion high-performance liquid chromatography for rapid examination of size differences of cereal proteins.

- Axford, D. W. E. ; McDermott, E. E. ; Redman, D. G. (1978). Small scale tests of bread making quality. *Milling, Feed and Fertiliser (Abbreviated as Mill. Feed Fertil.)*, 66, 18–21.
- Babay, E., Hanana, M., Mzid, R., Slim-Amara, H., Carrillo, J. M., & Rodríguez-Quijano, M. (2015). Influence of allelic prolamin variation and localities on durum wheat quality. *Journal of Cereal Science*, 63, 27–34. <https://doi.org/10.1016/j.jcs.2015.01.008>
- Barak, S., Mudgil, D., & Khatkar, B. S. (2013). Relationship of gliadin and glutenin proteins with dough rheology, flour pasting and bread making performance of wheat varieties. *LWT*, 51(1), 211–217. <https://doi.org/10.1016/j.lwt.2012.09.011>
- Barak, S., Mudgil, D., & Khatkar, B. S. (2015). Biochemical and Functional Properties of Wheat Gliadins: A Review. *Critical Reviews in Food Science and Nutrition*, 55(3), 357–368. <https://doi.org/10.1080/10408398.2012.654863>
- Beleggia, R., Platani, C., Nigro, F., & Cattivelli, L. (2010). A micro-method for the determination of Yellow Pigment Content in durum wheat. *Journal of Cereal Science*, 52(1), 106–110. <https://doi.org/10.1016/j.jcs.2010.03.008>
- Bettge, A. D., Morris, C. F., DeMacon, V. L., & Kidwell, K. K. (2002). Adaptation of AACC Method 56-11, Solvent Retention Capacity, for Use as an Early Generation Selection Tool for Cultivar Development. *Cereal Chemistry*, 79(5), 670–674. <https://doi.org/10.1094/CCHEM.2002.79.5.670>
- Blanco, A., Bellomo, M. P., Lotti, C., Maniglio, T., Pasqualone, A., Simeone, R., et al. (1998) Genetic mapping of sedimentation volume across environments using recombinant inbred lines of durum wheat. *Plant Breed* 117: 413–417.
- Blanco, A., Colasuonno, P., Gadaleta, A., Mangini, G., Schiavulli, A., Simeone, R., Digesù, A. M., De Vita, P., Mastrangelo, A. M., & Cattivelli, L. (2011). Quantitative trait loci for yellow pigment concentration and individual carotenoid compounds in durum wheat. *Journal of Cereal Science*, 54(2), 255–264. <https://doi.org/10.1016/j.jcs.2011.07.002>
- Blanco, A., Mangini, G., Giancaspro, A., Giove, S., Colasuonno, P., Simeone, R., Signorile, A., De Vita, P., Mastrangelo, A. M., Cattivelli, L., & Gadaleta, A. (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. *Molecular Breeding*, 30(1), 79–92. <https://doi.org/10.1007/s11032-011-9600-z>
- Boggini, G., & Pogna, N. E. (1989). The breadmaking quality and storage protein composition of Italian durum wheat. *Journal of Cereal Science*, 9(2), 131–138. [https://doi.org/10.1016/S0733-5210\(89\)80013-X](https://doi.org/10.1016/S0733-5210(89)80013-X)
- Borrelli, G. M., De Leonardis, A. M., Platani, C., & Troccoli, A. (2008). Distribution along durum wheat kernel of the components involved in semolina color. *Journal of Cereal Science*, 48(2), 494–502. <https://doi.org/10.1016/j.jcs.2007.11.007>
- Borrelli GM, Troccoli A, Di Fonzo N, Fares C (1999) Durum wheat lipoxygenase activity and other quality parameters that affect pasta colour. *Cereal Chemistry* 76, 335–340. doi:10.1094/CCHEM.1999.
- Bouabdellah, N., Chacón, E., Benavente, E., Ruiz, M., Giraldo, P., & Pascual, L. (2024). Image-assisted quantification of high and low molecular weight glutenin fractions in wheat by SDS-PAGE. *Journal of Cereal Science*, 118. <https://doi.org/10.1016/j.jcs.2024.103977>
- Branlard, G. P., & Metakovsky, E. V. (2006). Chapter 4 Some Gli Alleles Related to Common Wheat Dough Quality. In *Gliadin and Glutenin: The Unique Balance of Wheat Quality* (pp. 115–139). AACC International, Inc. <https://doi.org/10.1094/9781891127519.006>
- Brites, C., & Carrillo, J. M. (2001). Influence of high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits controlled by Glu-1 and Glu-3 loci on durum wheat quality. *Cereal Chemistry*, 78(1), 59–63. <https://doi.org/10.1094/CCHEM.2001.78.1.59>
- Brites, L. T. G. F., Schmiele, M., & Steel, C. J. (2018). Gluten-Free Bakery and Pasta Products. *Alternative and Replacement Foods*, 17, 385–410. <https://doi.org/10.1016/B978-0-12-811446-9.00013-7>
- Broccanello, C., Bellin, D., DalCorso, G., Furini, A., & Taranto, F. (2023). Genetic approaches to exploit landraces for improvement of *Triticum turgidum* ssp. durum in the age of climate change. In *Frontiers in Plant Science* (Vol. 14). Frontiers Media S.A. <https://doi.org/10.3389/fpls.2023.1101271>
- Bushuk, W., & Zillman, R. R. (1978). WHEAT CULTIVAR IDENTIFICATION BY GLIADIN ELECTROPHOREGRAMS. I. APPARATUS. METHOD AND

- NOMENCLATURE. *Canadian Journal of Plant Science*, 58(2), 505–515.
<https://doi.org/10.4141/cjps78-076>
- Cabas-Lühmann, P., Arriagada, O., Matus, I., Marcotuli, I., Gadaleta, A., & Schwember, A. R. (2023). Comparison of durum with ancient tetraploid wheats from an agronomical, chemical, nutritional, and genetic standpoints: a review. In *Euphytica* (Vol. 219, Issue 6). Springer Science and Business Media B.V. <https://doi.org/10.1007/s10681-023-03188-z>
- Campos, K. M., Royo, C., Schulthess, A., Villegas, D., Matus, I., Ammar, K., & Schwember, A. R. (2016). Association of phytoene synthase *Psy1-A1* and *Psy1-B1* allelic variants with semolina yellowness in durum wheat (*Triticum turgidum* L. var. durum). *Euphytica*, 207(1), 109–117. <https://doi.org/10.1007/s10681-015-1541-x>
- Carcea, M. (2021). Advances in understanding the nutritional value of lipids in wheat. In T. Beta (Ed.), *Improving the Nutritional and Nutraceutical Properties of Wheat and Other Cereals* (pp. 73–96). CABI.
- Carrera, A., Echenique, V., Zhang, W., Helguera, M., Manthey, F., Schrager, A., Picca, A., Cervigni, G., & Dubcovsky, J. (2007). A deletion at the *Lpx-B1* locus is associated with low lipoxygenase activity and improved pasta color in durum wheat (*Triticum turgidum* ssp. durum). *Journal of Cereal Science*, 45(1), 67–77. <https://doi.org/10.1016/j.jcs.2006.07.001>
- Carrillo, J. M., Martínez, M. C., Brites, C. M., Nieto-Taladriz, M. T., & Vázquez, J. F. (2000). Relationship between endosperm proteins and quality in durum wheat (*Triticum turgidum* L. var. durum). *Options Méditerranéennes*, 40, 463–467.
- Carrillo, J. M., Vazquez, J. F., & Orellana, J. (1990). Relationship Between Gluten Strength and Glutenin Proteins in Durum Wheat Cultivars. *Plant Breeding*, 104, 325–333.
- Cazzonelli, C. I., & Pogson, B. J. (2010). Source to sink: regulation of carotenoid biosynthesis in plants. In *Trends in Plant Science* (Vol. 15, Issue 5, pp. 266–274). <https://doi.org/10.1016/j.tplants.2010.02.003>
- Cecchini, C., Bresciani, A., Menesatti, P., Pagani, M. A., & Marti, A. (2021). Assessing the rheological properties of durum wheat semolina: A review. In *Foods* (Vol. 10, Issue 12). MDPI. <https://doi.org/10.3390/foods10122947>

- Chab, L., Biagini, L., & Severini, S. (2024). Towards an Effective Risk Management in Durum Wheat Production: A Systematic Review and Bibliometric Analysis of Factors Influencing Quality and Yield. In *Agriculture (Switzerland)* (Vol. 14, Issue 12). Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/agriculture14122266>
- Chacón, E. A., Vázquez, F. J., Giraldo, P., Carrillo, J. M., Benavente, E., & Rodríguez-Quijano, M. (2020). Allelic variation for prolamins in Spanish durum wheat landraces and its relationship with quality traits. *Agronomy*, 10(1). <https://doi.org/10.3390/agronomy10010136>
- Choi, I., Kang, C. S., Hyun, J. N., Lee, C. K., & Park, K. G. (2013). Mineral Compositions of Korean Wheat Cultivars. *Preventive Nutrition and Food Science*, 18(3), 214. <https://doi.org/10.3746/PNF.2013.18.3.214>
- Ciaffi, M., Benedettelli, S., Giorgi, B., Porceddu, E., & Lafiandra, D. (1991). Seed Storage Proteins of *Triticum turgidum* ssp. *dicoccoides* and Their Effect on the Technological Quality in Durum Wheat. *Plant Breeding*, 107, 309–319.
- Cinco-Moroyoqui, F. J., & MacRitchie, F. (2008). Quantitation of LMW-GS to HMW-GS ratio in wheat flours. *Cereal Chemistry*, 85(6), 824–829. <https://doi.org/10.1094/CCHEM-85-6-0824>
- Clarke, F. R., Clarke, J. M., McCaig, T. N., Knox, R. E., & DePauw, R. M. (2006). Inheritance of yellow pigment concentration in seven durum wheat crosses.
- Colasuonno, P., Gadaleta, A., Giancaspro, A., Nigro, D., Giove, S., Incerti, O., Mangini, G., Signorile, A., Simeone, R., & Blanco, A. (2014). Development of a high-density SNP-based linkage map and detection of yellow pigment content QTLs in durum wheat. *Molecular Breeding*, 34(4), 1563–1578. <https://doi.org/10.1007/s11032-014-0183-3>
- Colasuonno, P., Lozito, M. L., Marcotuli, I., Nigro, D., Giancaspro, A., Mangini, G., De Vita, P., Mastrangelo, A. M., Pecchioni, N., Houston, K., Simeone, R., Gadaleta, A., & Blanco, A. (2017). The carotenoid biosynthetic and catabolic genes in wheat and their association with yellow pigments. *BMC Genomics*, 18(1). <https://doi.org/10.1186/s12864-016-3395-6>
- Colasuonno, P., Marcotuli, I., Blanco, A., Maccaferri, M., Condorelli, G. E., Tuberosa, R., Parada, R., de Camargo, A. C., Schwember, A. R., & Gadaleta, A. (2019). Carotenoid Pigment Content in Durum Wheat (*Triticum turgidum* L. var *durum*): An Overview of Quantitative Trait Loci and

- Candidate Genes. In *Frontiers in Plant Science* (Vol. 10). Frontiers Media S.A. <https://doi.org/10.3389/fpls.2019.01347>
- Colasuonno, P., Marcotuli, I., Gadaleta, A., & Soriano, J. M. (2021). From genetic maps to qtl cloning: An overview for durum wheat. In *Plants* (Vol. 10, Issue 2, pp. 1–25). MDPI AG. <https://doi.org/10.3390/plants10020315>
- Collard, B. C. & Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1491), 557–572. <https://doi.org/10.1098/rstb.2007.2170>
- Cong, L., Wang, C., Chen, L., Liu, H., Yang, G., & He, G. (2009). Expression of phytoene synthase1 and carotene desaturase crt/ genes result in an increase in the total carotenoids content in transgenic elite wheat (*Triticum aestivum* L.). *Journal of Agricultural and Food Chemistry*, 57(18), 8652–8660. <https://doi.org/10.1021/jf9012218>
- Cornish, G. B. ; Bekes, F. ; Eagles, H. A. ; & Payne, P. I. (2006). Prediction of Dough Properties for Bread Wheats. In C. ; Wrigley, F. ; Békés, & W. Bushuk (Eds.), *Gliadin and Glutenin: The Unique Balance of Wheat Quality* (pp. 243–280). AACC Press. <https://doi.org/10.1094/9781891127519.012>
- Dai, S., Zhai, Z., Liu, Q., Yang, W., Liu, G., & Yan, Z. (2024). Quality and flour-blending effects of wheat lines with triple null alleles at the Glu-1 locus. *Journal of Food Composition and Analysis*, 129. <https://doi.org/10.1016/j.jfca.2024.106131>
- Day, L., Augustin, M. A., Batey, I. L., & Wrigley, C. W. (2006). Wheat-gluten uses and industry needs. In *Trends in Food Science and Technology* (Vol. 17, Issue 2, pp. 82–90). Elsevier Ltd. <https://doi.org/10.1016/j.tifs.2005.10.003>
- De Simone, V., Menzo, V., De Leonardis, A. M., Maria Ficco, D. B., Trono, D., Cattivelli, L., & De Vita, P. (2010). Different mechanisms control lipoxygenase activity in durum wheat kernels. *Journal of Cereal Science*, 52(2), 121–128. <https://doi.org/10.1016/j.jcs.2010.04.003>
- Desiderio, F. ; et al. (2021). Characterization of the Resistance to Powdery Mildew and Leaf Rust Carried by the Bread Wheat Cultivar Victo. *International Journal of Molecular Sciences*, 22(6), 3109. <https://doi.org/10.3390/ijms22063109>

- Dexter, J. E., Matsuo, R. R., Kosmolak, F. G., Leisle, D., & Marchylo, B. A. (1980). The suitability of the SDS-sedimentation test for assessing gluten strength in durum wheat. *Canadian Journal of Plant Science*, 60(1), 25–29. <https://doi.org/10.4141/CJPS80-004>
- Dhaka, V., & Khatkar, B. S. (2015). Effects of gliadin/glutenin and HMW-GS/LMW-GS ratio on dough rheological properties and bread-making potential of wheat varieties. *Journal of Food Quality*, 38(2), 71–82. <https://doi.org/10.1111/jfq.12122>
- Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M., & Robledo, C. W. (2020). InfoStat v2017 (2017). Centro de Transferencia InfoStat. <https://www.infostat.com.ar/>
- Dibari, B., Murat, F., Chosson, A., Gautier, V., Poncet, C., Lecomte, P., Mercier, I., Bergès, H., Pont, C., Blanco, A., & Salse, J. (2012). Deciphering the genomic structure, function and evolution of carotenogenesis related phytoene synthases in grasses. <http://www.biomedcentral.com/1471-2164/13/221>
- Dick, J. W. , & Quick, J. S. (1983). A modified screening test for rapid estimation of gluten strength in early-generation durum wheat breeding lines. *Cereal Chemistry*, 60.
- Diekmann, F. (2009). Commodity of the quarter: Wheat. *Journal of Agricultural and Food Information*, 10(4), 289–299. <https://doi.org/10.1080/10496500903245404>
- Dixon, J. (John M., & International Maize and Wheat Improvement Center. (2009). Wheat facts and futures 2009. CIMMYT.
- D'Ovidio, R., & Masci, S. (2004). The low-molecular-weight glutenin subunits of wheat gluten. *Journal of Cereal Science*, 39(3), 321–339. <https://doi.org/10.1016/j.jcs.2003.12.002>
- Doyle, J. J. ; Doyle, J. L. ; & Hortoriun, L. B. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12, 13–15.
- du Cros, D. L. (1987). Glutenin proteins and gluten strength in durum wheat. *Journal of Cereal Science*, 5(1), 3–12. [https://doi.org/10.1016/S0733-5210\(87\)80003-6](https://doi.org/10.1016/S0733-5210(87)80003-6)
- Elouafi, I., Nachit, M. M., & Martin, L. M. (2001). Identification of a microsatellite on chromosome 7B showing a strong linkage with yellow pigment in durum

- wheat (*Triticum turgidum* L. var. *durum*). *Hereditas*, 135(2–3), 255–261. <https://doi.org/10.1111/j.1601-5223.2001.t01-1-00255.x>
- EUROSTAT. European Union Statistical Database. Available online: <https://ec.europa.eu/eurostat/web/main/home> (accessed on 01 June 2023). (2023).
- FAOSTAT. <https://www.fao.org/faostat/fr/#data/QCL/visualize> (accessed on 01 May 2025). (2024).
- Feillet, P., Autran, J. C., & Icard-Vernière, C. (2000). Pasta brownness: An assessment. In *Journal of Cereal Science* (Vol. 32, Issue 3, pp. 215–233). Academic Press. <https://doi.org/10.1006/jcrs.2000.0326>
- Ferrante, P., Masci, S., D'Ovidio, R., Lafiandra, D., Volpi, C., & Mattei, B. (2006). A proteomic approach to verify in vivo expression of a novel γ -gliadin containing an extra cysteine residue. *Proteomics*, 6(6), 1908–1914. <https://doi.org/10.1002/pmic.200500236>
- Ficco, D. B. M., Mastrangelo, A. M., Trono, D., Borrelli, G. M., De Vita, P., Fares, C., Beleggia, R., Platani, C., & Papa, R. (2014). The colors of durum wheat: A review. In *Crop and Pasture Science* (Vol. 65, Issue 1, pp. 1–15). <https://doi.org/10.1071/CP13293>
- Fu, B. X., Chiremba, C., Pozniak, C. J., Wang, K., & Nam, S. (2017). Total phenolic and yellow pigment contents and antioxidant activities of durum wheat milling fractions. *Antioxidants*, 6(4). <https://doi.org/10.3390/antiox6040078>
- Fu, B. X., Schlichting, L., Pozniak, C. J., & Singh, A. K. (2011). A fast, simple, and reliable method to predict pasta yellowness. *Cereal Chemistry*, 88(3), 264–270. <https://doi.org/10.1094/CCHEM-12-10-0173>
- Fu, B. X., Schlichting, L., Pozniak, C. J., & Singh, A. K. (2013). Pigment loss from semolina to dough: Rapid measurement and relationship with pasta color. *Journal of Cereal Science*, 57(3), 560–566. <https://doi.org/10.1016/j.jcs.2013.03.007>
- Gallagher, C. E., Matthews, P. D., Li, F., & Wurtzel, E. T. (2004). Gene duplication in the carotenoid biosynthetic pathway preceded evolution of the grasses. *Plant Physiology*, 135(3), 1776–1783. <https://doi.org/10.1104/pp.104.039818>

- Garbus, I., Carrera, A. D., Dubcovsky, J., & Echenique, V. (2009). Physical mapping of durum wheat lipoxygenase genes. *Journal of Cereal Science*, 50(1), 67–73. <https://doi.org/10.1016/j.jcs.2009.02.008>
- García-Molina, M. D., Giménez, M. J., Sánchez-León, S., & Barro, F. (2019). Gluten free wheat: Are we there? In *Nutrients* (Vol. 11, Issue 3). MDPI AG. <https://doi.org/10.3390/nu11030487>
- Gianibelli, M. C., Larroque, O. R., MacRitchie, F., & Wrigley, C. W. (2001). Biochemical, genetic, and molecular characterization of wheat glutenin and its component subunits. In *Cereal Chemistry* (Vol. 78, Issue 6, pp. 635–646). American Association of Cereal Chemists. <https://doi.org/10.1094/CCHEM.2001.78.6.635>
- Giraldo, P. ; R. M. ; Ruiz, M. ; Ibba, M. I. ; Morris, C. F. ; Labuschagne, M. T. ; & Igrejas, G. (2020). Durum wheat storage protein composition and the role of LMW-GS in quality. In *Wheat Quality for Improving Processing and Human Health* (pp. 73–108).
- Giraldo, P., Royo, C., González, M., Carrillo, J. M., & Ruiz, M. (2016). Genetic diversity and association mapping for agromorphological and grain quality traits of a structured collection of durum wheat landraces including subsp. durum, turgidum and diccocon. *PLoS ONE*, 11(11). <https://doi.org/10.1371/journal.pone.0166577>
- Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K., & Delcour, J. A. (2005). Wheat flour constituents: How they impact bread quality, and how to impact their functionality. *Trends in Food Science and Technology*, 16(1–3), 12–30. <https://doi.org/10.1016/j.tifs.2004.02.011>
- Gojković Cvjetković, V. S., Grujić, R. D., Marjanović-Balaban, Ž. R., Stanojević, L. P., Stanojević, J. S., & Cakić, M. D. (2019). GLIADIN ANALYSIS BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. *Advanced Technologies*, 8(2), 30–36. <https://doi.org/10.5937/savteh1902030g>
- Grosse-Heilmann, M., Cristiano, E., Deidda, R., & Viola, F. (2024). Durum wheat productivity today and tomorrow: A review of influencing factors and climate change effects. In *Resources, Environment and Sustainability* (Vol. 17). Elsevier B.V. <https://doi.org/10.1016/j.resenv.2024.100170>

- Guedira, M., Xiong, M., Hao, Y. F., Johnson, J., Harrison, S., Marshall, D., & Brown-Guedira, G. (2016). Heading date QTL in winter wheat (*Triticum aestivum* L.) coincide with major developmental genes *VERNALIZATION1* and *PHOTOPERIOD1*. *PLoS ONE*, 11(5). <https://doi.org/10.1371/journal.pone.0154242>
- Guo, Y., Zhang, G., Guo, B., Qu, C., Zhang, M., Kong, F., Zhao, Y., & Li, S. (2020). QTL mapping for quality traits using a highdensity genetic map of wheat. *PLoS ONE*, 15(3). <https://doi.org/10.1371/journal.pone.0230601>
- Gupta, R. B. , Khan, K. , & Macritchie, F. ,. (1993). Biochemical Basis of Flour Properties in Bread Wheats. I. Effects of Variation in the Quantity and Size Distribution of Polymeric Protein. *J. Cereal. Sci.* <https://doi.org/10.1006/jcrs.1993.1031>.
- Guzmán, C., Ibba, M. I., Álvarez, J. B., Sissons, M., & Morris, C. (2022). Wheat Quality. In *Wheat Improvement: Food Security in a Changing Climate* (pp. 177–193). Springer International Publishing. https://doi.org/10.1007/978-3-030-90673-3_11
- Guzman, C., Peña, R. J., Singh, R., Autrique, E., Dreisigacker, S., Crossa, J., Rutkoski, J., Poland, J., & Battenfield, S. (2016). Wheat quality improvement at CIMMYT and the use of genomic selection on it. In *Applied and Translational Genomics* (Vol. 11, pp. 3–8). Elsevier B.V. <https://doi.org/10.1016/j.atg.2016.10.004>
- Guzmán, C., Posadas-Romano, G., Hernández-Espinosa, N., Morales-Dorantes, A., & Peña, R. J. (2015). A new standard water absorption criteria based on solvent retention capacity (SRC) to determine dough mixing properties, viscoelasticity, and bread-making quality. *Journal of Cereal Science*, 66, 59–65. <https://doi.org/10.1016/J.JCS.2015.10.009>
- Haraszi, R., Ikeda, T. M. , Pe~na, R. J. , & Branlard, G. ,. (2020). Gluten analysis. In G. , Igrejas, T. , Ikeda, & C. Guzm´an (Eds.), *Wheat Quality for Improving Processing and Human Health*. Springer, Chambridge (pp. 109–144).
- He, X. Y., He, Z. H., Ma, W., Appels, R., & Xia, X. C. (2009). Allelic variants of phytoene synthase 1 (*Psy1**) genes in Chinese and CIMMYT wheat cultivars and development of functional markers for flour color. *Molecular Breeding*, 23(4), 553–563. <https://doi.org/10.1007/s11032-009-9255-1>

- He, X. Y., Zhang, Y. L., He, Z. H., Wu, Y. P., Xiao, Y. G., Ma, C. X., & Xia, X. C. (2008). Characterization of phytoene synthase 1 gene (Psy1*) located on common wheat chromosome 7A and development of a functional marker. *Theoretical and Applied Genetics*, 116(2), 213–221. <https://doi.org/10.1007/s00122-007-0660-8>
- Helguera, M. ., (2020). Grain quality in breedin. . In G. , Igrejas, T. , Ikeda, C. Guzm'an, & (Eds.) (Eds.), *Wheat Quality for Improving Processing and Human Health*. (pp. 271–306).
- Hessler, T. G., Thomson, M. J., Benscher, D., Nachit, M. M., & Sorrells, M. E. (2002). Association of a Lipoxygenase Locus, Lpx-B1, with Variation in Lipoxygenase Activity in Durum Wheat Seeds. <https://doi.org/10.2135/cropsci2002.1695>
- Hoseney, R. C. (1994). *Principles of Cereal Science and Technology*. American Association of Cereal Chemists, Inc.
- Hoseney, R. C. , Finney, K. F., Shogren, M. D., Pomeranz, Y. (1971). Functional breadmaking and biochemical properties of wheat four components VII O .starch. 191–201.
- Howitt, C. A., Cavanagh, C. R., Bowerman, A. F., Cazzonelli, C., Rampling, L., Mimica, J. L., & Pogson, B. J. (2009). Alternative splicing, activation of cryptic exons and amino acid substitutions in carotenoid biosynthetic genes are associated with lutein accumulation in wheat endosperm. *Functional and Integrative Genomics*, 9(3), 363–376. <https://doi.org/10.1007/s10142-009-0121-3>
- Ibba, M. I. Kiszonas, A. M. Morris, C. F. (2017). Evidence of intralocus recombination at the Glu-3 loci in bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, 130, 891–902.
- Ikeda, T. M., Yanaka, M., & Takata, K. (2014). Comparison of quality-related alleles among Australian and North American wheat classes exported to Japan. *Cereal Chemistry*, 91(6), 616–622. <https://doi.org/10.1094/CCHEM-01-14-0009-R>
- International AACC. (2013). 14 Color and Pigments Methods. <https://www.cerealsgrains.org/resources/Methods/Pages/14ColorPigments.aspx>

- Jackson, E. A., Holt, L. M., & Payne, P. I. (1983). Characterisation of high molecular weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and the chromosomal localisation of their controlling genes. In *Theor Appl Genet* (Vol. 66). Springer-Verlag.
- Javid Iqbal, M., Shams, N., & Fatima, K. (2022). Nutritional Quality of Wheat. *Wheat*. <https://doi.org/10.5772/INTECHOPEN.104659>
- Johansson, E. (1996). Quality evaluation of D-zone omega gliadins in wheat. *Plant Breeding*, 115, 57–62.
- Johansson, E., Prieto-Linde, M. L., & Jönsson, J. O. (2001). Effects of wheat cultivar and nitrogen application on storage protein composition and breadmaking quality. *Cereal Chemistry*, 78(1), 19–25. <https://doi.org/10.1094/CCHEM.2001.78.1.19>
- Johnson, M., Kumar, A., Oladzad-Abbasabadi, A., Salsman, E., Aoun, M., Manthey, F. A., & Elias, E. M. (2019). Association mapping for 24 traits related to protein content, gluten strength, color, cooking, and milling quality using balanced and unbalanced data in durum wheat [*Triticum turgidum* L. Var. Durum (Desf.)]. *Frontiers in Genetics*, 10(JUL). <https://doi.org/10.3389/fgene.2019.00717>
- Johnston, R. A., Quick, J. S., & Hammond, J. J. (1983). Inheritance of Semolina Color in Six Durum Wheat Crosses 1. *Crop Science*, 23(4), 607–610. <https://doi.org/10.2135/cropsci1983.0011183x002300040001x>
- Jurkovic, Z., Sudar, R., Drezner, G., & Horvat, D. (2000). The HMW glutenin subunit composition of OS wheat cultivars and their relationship with bread-making quality. *Cereal Research Communications*, 28(3), 271–277. <https://doi.org/10.1007/BF03543604/METRICS>
- Kaan, F., Branlard, G., Chihab, B., & Borries, C. (1993). Relations between genes coding for grain storage protein and two pasta cooking quality criteria among durum wheat (*T. durum* Desf.) genetic resources. *Journal of Genetics and Breeding*, 47, 151–156.
- Kean, E. G., Bordenave, N., Ejeta, G., Hamaker, B. R., & Ferruzzi, M. G. (2011). Carotenoid bioaccessibility from whole grain and decorticated yellow endosperm sorghum porridge. *Journal of Cereal Science*, 54(3), 450–459. <https://doi.org/10.1016/j.jcs.2011.08.010>

- Khalid, A., Hameed, A., & Tahir, M. F. (2023). Wheat quality: A review on chemical composition, nutritional attributes, grain anatomy, types, classification, and function of seed storage proteins in bread making quality. *Frontiers in Nutrition*, 10, 1053196. <https://doi.org/10.3389/fnut.2023.1053196>
- Kilian, A. ;, Wenzl, P. ;, Huttner, E. ;, Carling, J. ;, Xia, L. ;, Blois, H. ;, & et al. (2012). Diversity Arrays Technology: A Generic Genome Profiling Technology on Open Platforms. *Methods in Molecular Biology*, 888, 67–89.
- Kiszonas, A. M., & Morris, C. F. (2018). Wheat breeding for quality: A historical review. In *Cereal Chemistry* (Vol. 95, Issue 1, pp. 17–34). Wiley-Blackwell. <https://doi.org/10.1094/CCHEM-05-17-0103-FI>
- Koga, S., Böcker, U., Moldestad, A., Tosi, P., Shewry, P. R., Mosleth, E. F., & Uhlen, A. K. (2016). Influence of temperature during grain filling on gluten viscoelastic properties and gluten protein composition. *Journal of the Science of Food and Agriculture*, 96(1), 122–130. <https://doi.org/10.1002/jsfa.7068>
- KOSAMBI, D. D. (1943). THE ESTIMATION OF MAP DISTANCES FROM RECOMBINATION VALUES. *Annals of Eugenics*, 12(1), 172–175. <https://doi.org/10.1111/j.1469-1809.1943.tb02321.x>
- Labuschagne, & M. T. Aucamp, U. (2004). The use of size exclusion high performance liquid chromatography (SE-HPLC) for wheat quality prediction in South Africa. *South African Journal of Plant and Soil*, 21(1), 8–12.
- Labuschagne, M. T., & Igrejas, G. (2020). Proteomics as a Tool in Gluten Protein Research. *Wheat Quality For Improving Processing And Human Health*, 145–169. https://doi.org/10.1007/978-3-030-34163-3_7
- Lachman, J., Martinek, P., Kotíková, Z., Orsák, M., & Šulc, M. (2017). Genetics and chemistry of pigments in wheat grain – A review. In *Journal of Cereal Science* (Vol. 74, pp. 145–154). Academic Press. <https://doi.org/10.1016/j.jcs.2017.02.007>
- Lafiandra, D. & Kasarda, D. D. (1985). One and two-dimensional (two-pH) polyacrylamide gel electrophoresis in a single gel: separation of wheat proteins. *Cereal Chemistry*, 62, 314–319.
- Laidò, G., Mangini, G., Taranto, F., Gadaleta, A., Blanco, A., Cattivelli, L., Marone, D., Mastrangelo, A. M., Papa, R., & De Vita, P. (2013). Genetic Diversity and

- Population Structure of Tetraploid Wheats (*Triticum turgidum* L.) Estimated by SSR, DArT and Pedigree Data. *PLoS ONE*, 8(6). <https://doi.org/10.1371/journal.pone.0067280>
- Lander, E. S., & Botsteins'b, D. + \$. (1989). Mapping Mendelian Factors Underlying Quantitative Traits Using RFLP Linkage Maps.
- Li, M., Yue, Q., Liu, C., Zheng, X., Hong, J., Li, L., & Bian, K. (2020). Effect of gliadin/glutenin ratio on pasting, thermal, and structural properties of wheat starch. *Journal of Cereal Science*, 93. <https://doi.org/10.1016/j.jcs.2020.102973>
- Liu, C.-Y. (1995). Identification of a New Low Mr Glutenin Subunit Locus on Chromosome 1B of Durum Wheat. In *dTournal of Cereal Science* (Vol. 21).
- Liu, D., Yang, H., Zhang, Z., Chen, Q., Guo, W., Rossi, V., Xin, M., Du, J., Hu, Z., Liu, J., Peng, H., Ni, Z., Sun, Q., & Yao, Y. (2023). An elite γ -gliadin allele improves end-use quality in wheat. *New Phytologist*, 239(1), 87–101. <https://doi.org/10.1111/nph.18722>
- Liu, D. ;, Zhang, X. ;, Jiang, W. ;, Li, M. ;, Wu, X. ;, Gao, D. ;, Bie, T. ;, & Lu, C. (2022). Influence of high-molecular-weight glutenin subunit deletions at the GLU-A1 and GLU-D1 loci on protein body development, protein components and dough properties of wheat (*Triticum aestivum* L.). *Journal of Integrative Agriculture*, 21, 1867–1876. [https://doi.org/10.1016/S2095-3119\(21\)63605-5](https://doi.org/10.1016/S2095-3119(21)63605-5)
- Liu, L. , Ikeda, T. M., Branlard, G., Peña, R. J., Rogers, W. J., Lerner, S. E., Kolman, M. A., Xia, X., Wang, L., Ma, W., Appels, R., Yoshida, H., Wang, A., Yan, Y., & He, Z. (2010). Comparison of low molecular weight glutenin subunits identified by SDS-PAGE, 2-DE, MALDI-TOF-MS and PCR in common wheat. *BMC Plant Biology*, 10. <https://doi.org/10.1186/1471-2229-10-124>
- López-Fernández, M., García-Abadillo, J., Uauy, C., Ruiz, M., Giraldo, P., & Pascual, L. (2023). Genome wide association in Spanish bread wheat landraces identifies six key genomic regions that constitute potential targets for improving grain yield related traits. *Theoretical and Applied Genetics*, 136(12). <https://doi.org/10.1007/s00122-023-04492-x>
- López-Fernández, M., Pascual, L., Faci, I., Fernández, M., Ruiz, M., Benavente, E., & Giraldo, P. (2021). Exploring the end-use quality potential of a collection

- of spanish bread wheat landraces. *Plants*, 10(4).
<https://doi.org/10.3390/plants10040620>
- Maccaferri, M., Cane, M. A., Sanguineti, M. C., Salvi, S., Colalongo, M. C., Massi, A., Clarke, F., Knox, R., Pozniak, C. J., Clarke, J. M., Fahima, T., Dubcovsky, J., Xu, S., Ammar, K., Karsai, I., Vida, G., & Tuberosa, R. (2014). A consensus framework map of durum wheat (*Triticum durum* Desf.) suitable for linkage disequilibrium analysis and genome-wide association mapping. *BMC Genomics*, 15(1), 1–21. <https://doi.org/10.1186/1471-2164-15-873/TABLES/1>
- Maccaferri, M., Harris, N. S., Twardziok, S. O., Pasam, R. K., Gundlach, H., Spannagl, M., Ormanbekova, D., Lux, T., Prade, V. M., Milner, S. G., Himmelbach, A., Mascher, M., Bagnaresi, P., Faccioli, P., Cozzi, P., Lauria, M., Lazzari, B., Stella, A., Manconi, A., ... Cattivelli, L. (2019). Durum wheat genome highlights past domestication signatures and future improvement targets. *Nature Genetics*, 51(5), 885–895. <https://doi.org/10.1038/s41588-019-0381-3>
- Maccaferri, M., Ricci, A., Salvi, S., Milner, S. G., Noli, E., Martelli, P. L., Casadio, R., Akhunov, E., Scalabrin, S., Vendramin, V., Ammar, K., Blanco, A., Desiderio, F., Distelfeld, A., Dubcovsky, J. ., Fahima, T., Faris, J., Korol, A., Massi, A., ... Tuberosa, R. (2015). A high-density, SNP-based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. *Plant Biotechnology Journal*, 13(5), 648–663. DOI: 10.1111/pbi.12288
- Magallanes-López, A. M., Ammar, K., Morales-Dorantes, A., González-Santoyo, H., Crossa, J., & Guzmán, C. (2017). Grain quality traits of commercial durum wheat varieties and their relationships with drought stress and glutenins composition. *Journal of Cereal Science*, 75, 1–9. <https://doi.org/10.1016/j.jcs.2017.03.005>
- Malik, P., Kumar, J., Singh, S., Sharma, S., Meher, P. K., Sharma, M. K., Roy, J. K., Sharma, P. K., Balyan, H. S., Gupta, P. K., & Sharma, S. (2021). Single-trait, multi-locus and multi-trait GWAS using four different models for yield traits in bread wheat. *Molecular Breeding*, 41(8). <https://doi.org/10.1007/s11032-021-01240-1>
- Manes, Y. (2006). Breeding for Yield and Quality. CIMMYT Workshop on Wheat Quality (This Replaces “Event Title”).

- Mangini, G. ;, Blanco, A. ;, Nigro, D. ;, Signorile, M. A. ;, & Simeone, R. (2021). Candidate Genes and Quantitative Trait Loci for Grain Yield and Seed Size in Durum Wheat. *Plants*, 10, 213. <https://doi.org/10.3390/plants10020312>
- Mangini, G., Nigro, D., Curci, P. L., Simeone, R., & Blanco, A. (2025). Genome-wide association study identifies QTL and candidate genes for grain size and weight in a *Triticum turgidum* collection. *Plant Genome*, 18(1). <https://doi.org/10.1002/tpg2.20562>
- Manna, F., Borrelli, G. M., Massardo, D. R., Wolf, K., Alifan, P., Giudice, L. Del, & Fonzo, N. Di. (1998). Differential expression of Lipoxygenase genes among durum wheat cultivars. In *Cereal Research Communications* (Vol. 26).
- Marchylo, B. A., Kruger, J. E., & Hatcher, D. W. (1989). Quantitative Reversed-phase High-performance Liquid Chromatographic Analysis of Wheat Storage Proteins as a Potential Quality Prediction Tool*. In *Journal of Cereal Science* (Vol. 9).
- Marcotuli, I., Soriano, J. M., & Gadaleta, A. (2022). A consensus map for quality traits in durum wheat based on genome-wide association studies and detection of ortho-meta QTL across cereal species. *Frontiers in Genetics*, 13. <https://doi.org/10.3389/fgene.2022.982418>
- Marcussen, T., Sandve, S. R., Heier, L., Spannagl, M., Pfeifer, M., Jakobsen, K. S., Wulff, B., Steuernagel, B., Mayer, K. F. X., Olsen, O. A., Rogers, J., Doležal, J., Pozniak, C., Eversole, K., Feuillet, C., Gill, B., Friebe, B., Lukaszewski, A. J., Sourdille, P., ... Praud, S. (2014). Ancient hybridizations among the ancestral genomes of bread wheat. *Science*, 345(6194). <https://doi.org/10.1126/science.1250092>
- Mares, D. J., & Campbell, A. W. (2001). Mapping components of flour and noodle color in Australian wheat. *Australian Journal of Agricultural Research*, 52(11–12), 1297–1309. <https://doi.org/10.1071/ar01048>
- Markgren, J., Hedenqvist, M., Rasheed, F., Skepö, M., & Johansson, E. (2020). Glutenin and Gliadin, a Piece in the Puzzle of their Structural Properties in the Cell Described through Monte Carlo Simulations. *Biomolecules* 2020, Vol. 10, Page 1095, 10(8), 1095. <https://doi.org/10.3390/BIOM10081095>
- Marone, D., Laidò, G., Gadaleta, A., Colasuonno, P., Ficco, D. B. M., Giancaspro, A., Giove, S., Panio, G., Russo, M. A., De Vita, P., Cattivelli, L., Papa, R., Blanco, A., & Mastrangelo, A. M. (2012). A high-density consensus map of A

- and B wheat genomes. *Theoretical and Applied Genetics*, 125(8), 1619–1638.
<https://doi.org/10.1007/s00122-012-1939-y>
- Martinez, M. D. C., Ruiz, M., & Carrillo, J. M. (2005). Effects of different prolamins alleles on durum wheat quality properties. *Journal of Cereal Science*, 41(1), 123–131. <https://doi.org/10.1016/j.jcs.2004.10.005>
- Martínez-Moreno, F., Ammar, K., & Solís, I. (2022). Global Changes in Cultivated Area and Breeding Activities of Durum Wheat from 1800 to Date: A Historical Review. In *Agronomy* (Vol. 12, Issue 5). MDPI. <https://doi.org/10.3390/agronomy12051135>
- Mcintosh, R. A., Yamazaki, Y., Dubcovsky, J. ., Rogers, W. J., Morris, C., Appels, R., & Xia, X. C. (2013). Catalogue of Gene Symbols for Wheat. Catalogue of Gene Symbols for Wheat (If Not Already Filled). <https://shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp>.
- Mejías, J. H., Lu, X., Osorio, C., Ullman, J. L., von Wettstein, D., & Rustgi, S. (2014). Analysis of wheat prolamins, the causative agents of celiac sprue, using reversed phase high performance liquid chromatography (RP-HPLC) and matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). *Nutrients*, 6(4), 1578–1597. <https://doi.org/10.3390/nu6041578>
- Metakovsky, E., Melnik, V. A., Pascual, L., & Wrigley, C. W. (2024). How Important Are Genetic Diversity and Cultivar Uniformity in Wheat? The Case of Gliadins. In *Genes* (Vol. 15, Issue 7). Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/genes15070927>
- Metakovsky, E., Melnik, V., Pascual, L., & Wrigley, C. W. (2020). Over 40% of 450 registered wheat cultivars (*Triticum aestivum*) worldwide are composed of multiple biotypes. *Journal of Cereal Science*, 96. <https://doi.org/10.1016/j.jcs.2020.103088>
- Metakovsky, E. V., & Novoselskaya, A. Y. (1991). Gliadin allele identification in common wheat. 1. Methodological aspects of the analysis of gliadin pattern by one-dimensional polyacrylamide gel electrophoresis. *Journal of Genetics and Breeding (Abbreviated as J. Genet. & Breed.)*, 45, 317–324.
- Metakovsky, E. V., Novoselskaya, A. Y., Kopus, M. M., Sobko, T. A., & Sozinov, A. A. (1984). Blocks of gliadin components in winter wheat detected by one-

- dimensional polyacrylamide gel electrophoresis. In *Theor Appl Genet* (Vol. 67). Springer-Verlag.
- Morris, C. F. (2015). Evaluation of Wheat-Grain Quality Attributes. In *Encyclopedia of Food Grains: Second Edition* (Vols. 3–4, pp. 251–256). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-394437-5.00247-3>
- Morris, C. F., Paszczynska, B., Bettge, A. D., King, G.E. (2007). A critical examination of the sodium dodecyl sulfate (SDS) sedimentation test for wheat meals. *Journal of the Science of Food and Agriculture*, 87(4), 607–615.
- Morrison, W. R. (1988). Wheat: Chemistry and Technology (Y. Pomeranz, Ed.; pp. 373–439). American Association of Cereal Chemists.
- Nazco, R., Peña, R. J., Ammar, K., Villegas, D., Crossa, J., & Royo, C. (2014). Durum wheat (*Triticum durum* Desf.) Mediterranean landraces as sources of variability for allelic combinations at Glu-1/Glu-3 loci affecting gluten strength and pasta cooking quality. *Genetic Resources and Crop Evolution*, 61(6), 1219–1236. <https://doi.org/10.1007/s10722-014-0104-7>
- Nesbitt, M., & Samuel, D. (1996). From staple crop to extinction? The archaeology and history of the hulled wheats.
- Nigro, D., Monaci, L., Pilolli, R., & Gadaleta, A. (2020). Allelic variation of gliadin-encoding genes in a collection of tetraploid wheat genotypes. *Cereal Research Communications*, 48(4), 507–515. <https://doi.org/10.1007/s42976-020-00061-w>
- Nishino, H., Murakoshi, M., Tokuda, H., & Satomi, Y. (2009). Cancer prevention by carotenoids. In *Archives of Biochemistry and Biophysics* (Vol. 483, Issue 2, pp. 165–168). <https://doi.org/10.1016/j.abb.2008.09.011>
- Nunes-Miranda, J. D., Bancel, E., Viala, D., Chambon, C., Capelo, J. L., Branlard, G., Ravel, C., & Igrejas, G. (2017). Wheat glutenin: the “tail” of the 1By protein subunits. *Journal of Proteomics*, 169, 136–142. <https://doi.org/10.1016/j.jprot.2017.05.019>
- Oak, M. D., Tamhankar, S. A., Rao, V. S., & Bhosale, S. B. (2004). Relationship of HMW, LMW Glutenin Subunits and γ -gliadins with Gluten Strength in Indian Durum Wheats. In *J. Plant Biochemistry & Biotechnology* (Vol. 13).

- Padhy, A. K., Kaur, P., Singh, S., Kashyap, L., & Sharma, A. (2022). Colored wheat and derived products: key to global nutritional security. In *Critical Reviews in Food Science and Nutrition*. Taylor and Francis Ltd. <https://doi.org/10.1080/10408398.2022.2119366>
- Pagnotta, M. A.; Blanco, A.; Gadaleta, A.; & Fares, C. (2005). Functional determinants of grain quality. In C.; Royo, M. M.; Nachit, N.; Di Fonzo, J. L.; Araus, W. H.; Pfeiffer, & G. A. Slafer (Eds.), *Durum wheat breeding: current approaches and future strategies*. Vol. 1 (Vol. 1, pp. 483–527). Food Products Press.
- Panozzo, J. F., & Eagles, H. A. (2000). Cultivar and environmental effects on quality characters in wheat. II. Protein. *Australian Journal of Agricultural Research*, 51(5), 629–636. <https://doi.org/10.1071/AR99137>
- Parada, R., Royo, C., Gadaleta, A., Colasuonno, P., Marcotuli, I., Matus, I., Castillo, D., de Camargo, A. C., Araya-Flores, J., Villegas, D., & Schwember, A. R. (2020). Phytoene synthase 1 (Psy-1) and lipoxygenase 1 (lpx-1) genes influence on semolina yellowness in wheat mediterranean germplasm. *International Journal of Molecular Sciences*, 21(13), 1–22. <https://doi.org/10.3390/ijms21134669>
- Parker, G. D., Chalmers, · K J, Langridge, P., Rathjen, A. J., & Rathjen, · A J. (1998). Mapping loci associated with flour color in wheat (*Triticum aestivum* L.). In *Theor Appl Genet* (Vol. 97). Springer-Verlag.
- Pasha, I., Anjum, F. M., & Morris, C. F. (2010). Grain Hardness: A Major Determinant of Wheat Quality. *Food Science and Technology International*, 16(6), 511–522. <https://doi.org/10.1177/1082013210379691>
- Pasten, M. C., Roncallo, P. F., Acosta, E. Y. C., Echenique, V., & Garbus, I. (2021). Association of novel characterized sequence variations in the ζ -carotene desaturase (Zds) gene with yellow color and yellow pigment content in durum wheat cultivars. *Journal of Cereal Science*, 99, 103185.
- Patil, R. M., Oak, M. D., Tamhankar, S. A., Sourdille, P., & Rao, V. S. (2008). Mapping and validation of a major QTL for yellow pigment content on 7AL in durum wheat (*Triticum turgidum* L. ssp. durum). *Molecular Breeding*, 21(4), 485–496. <https://doi.org/10.1007/s11032-007-9147-1>

- Payne, P. I. . (1987). Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annual Review of Plant Physiology*, 38, 141–153. <https://doi.org/10.1146/annurev. pp.38.060187.001041>.
- Payne, P. I., Holt, L. M., & Law, C. N. (1981). Structural and Genetical Studies on the High-molecular-weight Subunits of Wheat Glutenin Part 1 9 Allelic Variation in Subunits Amongst Varieties of Wheat (*Triticum aestivum*). In *Theor. Appl. Genet* (Vol. 60).
- Payne, P. I., Holt, L. M., Lawrence, G. J., & Law, C. N. (1982). The genetics of gliadin and glutenin, the major storage proteins of the wheat endosperm. *Plant Foods for Human Nutrition*, 31, 229–241.
- Payne, P. I., & Lawrence, G. J. (1983). Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1, and Glu-D1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat. In *Research Communications* (Vol. 11, Issue 1). <https://about.jstor.org/terms>
- Payne, P. I., Nightingale, M. A., Krattiger, A. F., & Holt, L. M. (1987). The Relationship between HMW Glutenin Subunit Composition and the Bread-making Quality of British-grown Wheat Varieties. In *J. Sci. Food Agric* (Vol. 40).
- Payne, P. I., Seekings, J. A., Worland, A. J., Jarvis, M. G., & Holt, L. M. (1987). Allelic variation of Glutenin Subunits and Gliadins and its Effect on Breadmaking Quality in Wheat: Analysis of F s Progeny from Chinese Spring x Chinese Spring (Hope 1A). In *Journal of Cereal Science* (Vol. 6).
- Peña, R. J. (2000). Durum wheat for pasta and bread making. Comparison of methods used in breeding to determine gluten quality-related parameters. In C. Royo, M. M. Nachit, N. Di Fonzo, & J. L. Araus (Eds.), *Durum Wheat Improvement in the Mediterranean Region: New Challenges* (pp. 423–430). Instituto Agronomico Mediterraneo de Zaragoza.
- Peña, R. J., Trethowan, R., Pfeiffer, W. H., & Van Ginkel, M. (2002). Quality (End-Use) improvement in wheat: Compositional, genetic, and environmental factors. In *Journal of Crop Production* (Vol. 5, Issues 1–2, pp. 1–37). https://doi.org/10.1300/J144v05n01_02
- Peña, R. J. (2008). Wheat for bread and other foods. <https://www.fao.org/4/y4011e/y4011e0w.htm>

- Peña-Bautista, R. J., Hernandez-Espinosa, N., Jones, J. M., Guzman, C., & Braun, H. J. (2017). CIMMYT series on carbohydrates, wheat, grains, and health: Wheat-based foods: Their global and regional importance in the food supply, nutrition, and health. *Cereal Foods World*, 62(5), 231–249.
- Peters Haugrud, A. R., Achilli, A. L., Martínez-Peña, R., & Klymiuk, V. (2024). Future of durum wheat research and breeding: Insights from early career researchers. In *Plant Genome*. John Wiley and Sons Inc. <https://doi.org/10.1002/tpg2.20453>
- Petersen, G., Seberg, O., Yde, M., & Berthelsen, K. (2006). Phylogenetic relationships of *Triticum* and *Aegilops* and evidence for the origin of the A, B, and D genomes of common wheat (*Triticum aestivum*). *Molecular Phylogenetics and Evolution*, 39(1), 70–82. <https://doi.org/10.1016/j.ympev.2006.01.023>
- Plessis, A., Ravel, C., Bordes, J., Balfourier, F., & Martre, P. (2013). Association study of wheat grain protein composition reveals that gliadin and glutenin composition are trans-regulated by different chromosome regions. *Journal of Experimental Botany*, 64(12), 3627–3644. <https://doi.org/10.1093/jxb/ert188>
- Pogna, N. E., Autran, J. C., Mellini, F., Lafiandra, D., & Feillet, P. (1990). Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: Genetics and relationship to gluten strength. *Journal of Cereal Science*, 11(1), 15–34. [https://doi.org/10.1016/S0733-5210\(09\)80178-1](https://doi.org/10.1016/S0733-5210(09)80178-1)
- Popineau, Y., Cornec, M., Lefebvre, J., & Marchylo, B. (1994). Influence of High Mr Glutenin Subunits on Glutenin Polymers and Rheological Properties of Glutens and Gluten Subfractions of Near-isogenic Lines of Wheat Sicco. *Journal of Cereal Science*, 19(3), 231–241.
- Porceddu, E., Turchetta, T., Masci, S., Impigld, A., & Nachit, M. (1998a). Wheat: Prospects for Global Improvement.
- Porceddu, E., Turchetta, T., Masci, S., D'Ovidio, R., Lafiandra, D., Kasarda, D. D., & et al. (1998b). Variation in endosperm protein composition and technological quality properties in durum wheat. *Euphytica*, 100, 197–205.
- Pozniak, C. J., Knox, R. E., Clarke, F. R., & Clarke, J. M. (2007). Identification of QTL and association of a phytoene synthase gene with endosperm color in

- durum wheat. *Theoretical and Applied Genetics*, 114(3), 525–537. <https://doi.org/10.1007/s00122-006-0453-5>
- R Core Team. (2022). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Raciti, C. N., Doust, M. A., Lombardo, G. M., Boggini, G., & Pecetti, L. (2003). Characterization of durum wheat mediterranean germplasm for high and low molecular weight glutenin subunits in relation with quality. *European Journal of Agronomy*. [https://doi.org/10.1016/S1161-0301\(02\)00091-6](https://doi.org/10.1016/S1161-0301(02)00091-6)
- Rasheed, A., Wen, W., Gao, F., Zhai, S., Jin, H., Liu, J., . . . Mather, D. E. (2016). Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. *Theoretical and Applied Genetics*, 129, 1843–1860. <https://doi.org/10.1007/s00122-016-2743-x>
- Ribeiro, M., Nunes, F. M., Guedes, S., Domingues, P., Silva, A. M., Carrillo, J. M., Rodriguez-Quijano, M., Branlard, G., & Igrejas, G. (2015). Efficient chemo-enzymatic gluten detoxification: Reducing toxic epitopes for celiac patients improving functional properties. *Scientific Reports*, 5. <https://doi.org/10.1038/srep18041>
- Rodriguez-Amaya. (2004). *HarvestPlus Handbook for Carotenoid Analysis (Vol. 2, HarvestPlus Series) (Vol. 2)*. IFPRI & CIAT (International Food Policy Research Institute & International Center for Tropical Agriculture).
- Roncallo, P. F., Cervigni, G. L., Jensen, C., Miranda, R., Carrera, A. D., Helguera, M., & Echenique, V. (2012). QTL analysis of main and epistatic effects for flour color traits in durum wheat. *Euphytica*, 185(1), 77–92. <https://doi.org/10.1007/s10681-012-0628-x>
- Roncallo, P. F. ;, Guzmán, C. ;, Larsen, A. O. ;, Achilli, A. L. ;, Dreisigacker, S. ;, Molfese, E. ;, Astiz, V. ;, & Echenique, V. (2021). Allelic Variation at Glutenin Loci (Glu-1, Glu-2 and Glu-3) in a Worldwide Durum Wheat Collection and Its Effect on Quality Attributes. *Foods*, 10(11), 2845. <https://doi.org/10.3390/foods10112845>
- Rubianes, J. (2007). *Prolaminas y marcadores moleculares relacionados con la calidad en trigo duro [Doctoral Thesis]*. Universidad Politécnica de Madrid.
- Ruiz M., & Carrillo. J. M. (1995). Relationships between different prolamin proteins and some quality properties in durum wheat. *Plant Breeding*, 114, 40–44. <https://doi.org/10.1111/j.1439-0523.1995.tb00756.x>

- Ruiz, M., & Carrillo, J. M. (1993). Linkage relationships between prolamin genes on chromosomes 1A and 1B of durum wheat. In *Theor Appl Genet* (Vol. 87). Springer-Verlag.
- Ruiz M, Carrillo JM (1996) Gli-B3/Glu-B2 encoded prolamins do not affect selected quality properties in the durum wheat cross 'Abadía'×'Mexicali 75'. *Plant Breeding* 115: 410–412.
- Ruiz, M., & Giraldo, P. (2021). The influence of allelic variability of prolamins on gluten quality in durum wheat: An overview. In *Journal of Cereal Science* (Vol. 101). Academic Press. <https://doi.org/10.1016/j.jcs.2021.103304>
- Russo, M. A., Ficco, D. B. M., Laidò, G., Marone, D., Papa, R., Blanco, A., Gadaleta, A., De Vita, P., & Mastrangelo, A. M. (2014). A dense durum wheat × T. dicoccum linkage map based on SNP markers for the study of seed morphology. *Molecular Breeding*, 34(4), 1579–1597. <https://doi.org/10.1007/s11032-014-0181-5>
- Saini, P., Kumar, N., Kumar, S., Mwaurah, P. W., Panghal, A., Attkan, A. K., Singh, V. K., Garg, M. K., & Singh, V. (2020). Bioactive compounds, nutritional benefits and food applications of colored wheat: a comprehensive review. In *Critical Reviews in Food Science and Nutrition* (pp. 1–14). Taylor and Francis Inc. <https://doi.org/10.1080/10408398.2020.1793727>
- Sansaloni, C., Petroli, C., Jaccoud, D., Carling, J., Detering, F., Grattapaglia, D., & Kilian, A. (2011). Diversity Arrays Technology (DArT) and next-generation sequencing combined: genome-wide, high throughput, highly informative genotyping for molecular breeding of Eucalyptus. *BMC Proceedings*, 5(S7). <https://doi.org/10.1186/1753-6561-5-s7-p54>
- Sarkar, A., & Fu, B. X. (2022). Impact of Quality Improvement and Milling Innovations on Durum Wheat and End Products. In *Foods* (Vol. 11, Issue 12). MDPI. <https://doi.org/10.3390/foods11121796>
- Schmid, M., Wieser, H., & Koehler, P. (2017). Disulphide structure of high-molecular-weight (HMW-) gliadins as affected by terminators. *Journal of Cereal Science*, 78, 66–74. <https://doi.org/10.1016/j.jcs.2016.12.007>
- Seib, P. A. (1994). Structure and Properties. In *Oyo Toshitsu Kagaku* (Vol. 41, Issue 1).
- Sharma, A., Garg, S., Sheikh, I., Vyas, P., & Dhaliwal, H. S. (2020). Effect of wheat grain protein composition on end-use quality. In *Journal of Food Science and*

- Technology (Vol. 57, Issue 8, pp. 2771–2785). Springer.
<https://doi.org/10.1007/s13197-019-04222-6>
- Shewry, P. (2019). What is gluten—Why is it special? In *Frontiers in Nutrition* (Vol. 6). Frontiers Media S.A. <https://doi.org/10.3389/fnut.2019.00101>
- Shewry, P. R. (2009). Wheat. In *Journal of Experimental Botany* (Vol. 60, Issue 6, pp. 1537–1553). <https://doi.org/10.1093/jxb/erp058>
- Shewry, P. R., & Belton, P. S. (2024). What do we really understand about wheat gluten structure and functionality? In *Journal of Cereal Science* (Vol. 117). Academic Press. <https://doi.org/10.1016/j.jcs.2024.103895>
- Shewry, P. R., & Halford, N. G. (2002). Cereal seed storage proteins: structures, properties and role in grain utilization. <http://jxb.oxfordjournals.org/>
- Shewry, P. R., Halford, N. G., & Lafiandra, D. (2003). Genetics of Wheat Gluten Proteins. *Advances in Genetics*, 49, 111–184. [https://doi.org/10.1016/S0065-2660\(03\)01003-4](https://doi.org/10.1016/S0065-2660(03)01003-4)
- Shewry, P. R., & Hey, S. J. (2015). The contribution of wheat to human diet and health. In *Food and Energy Security* (Vol. 4, Issue 3, pp. 178–202). Wiley-Blackwell Publishing Ltd. <https://doi.org/10.1002/FES3.64>
- Shewry, P. R., Napier, J. A., & Tatham, A. S. (1995). Seed Storage Proteins: Structures and Biosynthesis. In *The Plant Cell* (Vol. 7). American Society of Plant Physiologists.
- Shewry, P. R., Popineau, Y., Lafiandra, D., & Belton, P. (2001). Wheat glutenin subunits and dough elasticity: findings of the EUROWHEAT project.
- Siedow, J. N. (1991). PLANT LIPOXYGENASE: STRUCTURE AND FUNCTION. In *Annu. Rev. Plant Physiol. Plant Mol. Biol* (Vol. 42). www.annualreviews.org
- Singh, A., Reimer, S., Pozniak, C. J., Clarke, F. R., Clarke, J. M., Knox, R. E., & Singh, A. K. (2009). Allelic variation at Psy1-A1 and association with yellow pigment in durum wheat grain. *Theoretical and Applied Genetics*, 118(8), 1539–1548. <https://doi.org/10.1007/s00122-009-1001-x>
- Singh, N. K., Donovan, R., & Macritchie, F. (1990). Use of sonication and size-exclusion HPLC in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of bread making quality. *Cereal Chemistry*, 67, 161–170.

- Singh, N. K., & Shepherd, K. W. (1988). Linkage mapping of genes controlling endosperm storage proteins in wheat* 1. Genes on the short arms of group 1 chromosomes.
- Singh, N. K., Shepherd, K. W., & Cornish, G. B. (1991). A simplified SDS—PAGE procedure for separating LMW subunits of glutenin. *Journal of Cereal Science*, 14(3), 203–208. [https://doi.org/10.1016/S0733-5210\(09\)80039-8](https://doi.org/10.1016/S0733-5210(09)80039-8)
- Sissons, M. (2004). “Pasta,” in *Encyclopedia of grain science*. Eds. C. Wrigley, H. Corke, and C. Walker (London: Elsevier Academic Press), 409–418. doi: 10.1016/B0-12-765490-9/00123-3
- Sissons, M. (2008). Role of Durum Wheat Composition on the Quality of Pasta and Bread. *Food*, 2(2), 75–90. <https://www.researchgate.net/publication/228487489>.
- Sissons, M. (2022). Durum Wheat Products—Recent Advances. In *Foods* (Vol. 11, Issue 22). MDPI. <https://doi.org/10.3390/foods11223660>
- Sissons, M. J., Ames, N. P., Hare, R. A., & Clarke, J. M. (2005). Relationship between glutenin subunit composition and gluten strength measurements in durum wheat. *Journal of the Science of Food and Agriculture*, 85(14), 2445–2452. <https://doi.org/10.1002/jsfa.2272>
- Song, L., Li, L., Zhao, L., Liu, Z., Xie, T., & Li, X. (2020). Absence of Dx2 at Glu-D1 locus weakens gluten quality potentially regulated by expression of nitrogen metabolism enzymes and glutenin-related genes in wheat. *International Journal of Molecular Sciences*, 21(4). <https://doi.org/10.3390/ijms21041383>
- Soriano, J. M., Malosetti, M., Roselló, M., Sorrells, M. E. & Royo, C. Dissecting the old Mediterranean durum wheat genetic architecture for phenology, biomass and yield formation by association mapping and QTL meta-analysis. *PLoS One* 12, e0178290, <https://doi.org/10.1371/journal.pone.0178290> (2017).
- Soriano, J. M., & Alvaro, F. (2019). Discovering consensus genomic regions in wheat for root-related traits by QTL meta-analysis. *Scientific Reports*, 9(1). <https://doi.org/10.1038/s41598-019-47038-2>
- Storlie, E. W., Ihry, R. J., Baehr, L. M., Tieszen, K. A., Engbers, J. H., Anderson-Daniels, J. M., Davis, E. M., Gilbertson, A. G., Harden, N. R., Harris, K. A., Johnson, A. J., Kerkvleit, A. M., Moldan, M. M., Bell, M. E., & Wanous, M. K. (2009). Genomic regions influencing gene expression of the HMW

- glutenins in wheat. *Theoretical and Applied Genetics*, 118(2), 295–303. <https://doi.org/10.1007/s00122-008-0899-8>
- Subira, J., Peña, R. J., Álvaro, F., Ammar, K., Ramdani, A., & Royo, C. (2014). Breeding progress in the pasta-making quality of durum wheat cultivars released in Italy and Spain during the 20th Century. *Crop and Pasture Science*, 65(1), 16–26. <https://doi.org/10.1071/CP13238>
- Suchy, J., Lukow, O. M., Brown, D., DePauw, R., Fox, S., & Humphreys, G. (2007). Rapid assessment of glutenin and gliadin in wheat by UV spectrophotometer. *Crop Science*, 47(1), 91–99. <https://doi.org/10.2135/cropsci2006.05.0344>
- Taranto, F., Esposito, S., & De Vita, P. (2023a). Genomics for Yield and Yield Components in Durum Wheat. In *Plants* (Vol. 12, Issue 13). Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/plants12132571>
- Taranto, F., Esposito, S., Fania, F., Sica, R., Marzario, S., Logozzo, G., Gioia, T., & De Vita, P. (2023b). Breeding effects on durum wheat traits detected using GWAS and haplotype block analysis. *Frontiers in Plant Science*, 14. <https://doi.org/10.3389/fpls.2023.1206517>
- Tosi, P., Parker, M., Gritsch, C. S., Carzaniga, R., Martin, B., & Shewry, P. R. (2009). Trafficking of storage proteins in developing grain of wheat. *Journal of Experimental Botany*, 60(3), 979–991. <https://doi.org/10.1093/jxb/ern346>
- Trad, H., Ayed, S., Rhazi, L., Slim, A., Teixeira da Silva, J. A., Hellal, R., Sghaier, M., & Amara, H. S. (2014). Comparative quality analysis of gluten strength and the relationship with high molecular weight glutenin subunits of 6 Tunisian durum wheat genotypes. *Food Science and Biotechnology*, 23(5), 1363–1370. <https://doi.org/10.1007/s10068-014-0187-0>
- Troccoli, A., Ficco, D. B. M., Platani, C., D'Egidio, M. G., & Borrelli, G. M. (2025). Prediction of Pasta Colour Considering Traits Involved in Colour Expression of Durum Wheat Semolina. *Foods*, 14(3). <https://doi.org/10.3390/foods14030392>
- Trono, D., Pastore, D., & Fonzo, N. Di. (1999). Carotenoid Dependent Inhibition of Durum Wheat Lipxygenase. In *Journal of Cereal Science* (Vol. 29). <http://www.idealibrary.comon>

- Turchetta, T., Ciaffi, M., Porceddu, E., & Lafiandra, D. (1995). Relationship between electrophoretic pattern of storage proteins and gluten strength in durum wheat landraces from Turkey. *Plant Breeding*, 114, 406–412.
- Uthayakumaran, S., Stoddard, F. L., Gras, P. W., & Bekes, F. (2000). Effects of incorporated glutenins on functional properties of wheat dough. *Cereal Chemistry*, 77(6), 737–743. <https://doi.org/10.1094/CCHEM.2000.77.6.737>
- Vázquez, J. F., Ruiz, M., Nieto-Taladriz, M. T., & Albuquerque, M. M. (1996). Effects on Gluten Strength of Low Mr Glutenin Subunits Coded by Alleles at Glu-A3 and Glu-B3 Loci in Durum Wheat. In *Journal of Cereal Science* (Vol. 24).
- Van Ooijen JW, K. B. (2009). MapQTL 6. Software for the mapping of quantitative trait loci in experimental populations of diploid species.
- Van Ooijen JW (2019) JoinMap 5 ® Software for the calculation of genetic linkage maps in experimental populations of diploid species. *Kyazma B.V., Wageningen*, pp 1–61
- Vargas, V. H., Schulthess, A., Royo, C., Matus, I., & Schwember, A. R. (2016). Transcripts levels of Phytoene synthase 1 (Psy-1) are associated to semolina yellowness variation in durum wheat (*Triticum turgidum* L. ssp. durum). *Journal of Cereal Science*, 68, 155–163. <https://doi.org/10.1016/j.jcs.2016.01.011>
- Verlotta, A., De Simone, V., Mastrangelo, A. M., Cattivelli, L., Papa, R., & Trono, D. (2010). Insight into durum wheat Lpx-B1: A small gene family coding for the lipoxygenase responsible for carotenoid bleaching in mature grains. *BMC Plant Biology*, 10. <https://doi.org/10.1186/1471-2229-10-263>
- Viviani, A., Haile, J. K., Fernando, W. G. D., Ceoloni, C., Kuzmanović, L., Lhamo, D., Gu, Y. Q., Xu, S. S., Cai, X., Buerstmayr, H., Elias, E. M., Confortini, A., Bozzoli, M., Brar, G. S., Ruan, Y., Berraies, S., Hamada, W., Oufensou, S., Jayawardana, M., ... Cattivelli, L. (2025). Priority actions for Fusarium head blight resistance in durum wheat: Insights from the wheat initiative. In *Plant Genome* (Vol. 18, Issue 1). John Wiley and Sons Inc. <https://doi.org/10.1002/tpg2.20539>
- Voorrips, R. (2002). MapChart: software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, 93(1), 77–78. [10.1093/jhered/93.1.77](https://doi.org/10.1093/jhered/93.1.77)

- Voorrips, R. E. (2009). Computer Note MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. <http://www.intl-pag.org/>
- Wang, Z., Li, Y., Yang, Y., Liu, X., Qin, H., Dong, Z., Zheng, S., Zhang, K., & Wang, D. (2017). New insight into the function of wheat glutenin proteins as investigated with two series of genetic mutants. *Scientific Reports*, 7(1). <https://doi.org/10.1038/s41598-017-03393-6>
- Watson, A. ; G. S. ; W. M. J. ; et al., Ghosh, S. ;, Williams, M. J. ;, & et al. (2018). Speed breeding is a powerful tool to accelerate crop research and breeding. *Nature Plants*, 4, 23–29. <https://doi.org/10.1038/s41477-017-0083-8>
- Weegels, P. L., Hamer, R. J., & Schofield, J. D. (1996). Critical Review Functional Properties of Wheat Glutenin. In *Journal of Cereal Science* (Vol. 23).
- Whan, A. M., A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., Lillemo, M., Mather, D., Appels, R., . . . Akhunov, E., International Wheat Genome Sequencing Consortium. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, 12(6), 787–796. <https://doi.org/10.1111/pbi.12183>
- Wieser, H. , W. Bushuk, & F. MacRitchie. (2006). Gliadin and Glutenin: The Unique Balance of Wheat Quality. In C. Wrigley, F. Békés, & W. Bushuk (Eds.), *The polymeric glutenins*. (pp. 213–240). <https://doi.org/10.1094/9781891127519.010>.
- Wieser, H., Antes, S., & Seilmeier, W. (1998). Quantitative determination of gluten protein types in wheat flour by reversed-phase high-performance liquid chromatography. *Cereal Chemistry*, 75(5), 644–650. <https://doi.org/10.1094/CCHEM.1998.75.5.644>
- Wieser, H., & Kieffer, R. (2001). Correlations of the amount of gluten protein types to the technological properties of wheat flours determined on a micro-scale. *Journal of Cereal Science*, 34(1), 19–27. <https://doi.org/10.1006/jcrs.2000.0385>
- Wieser, H., Koehler, P., & Scherf, K. A. (2020a). Chemical composition of wheat grains. In *Wheat - An Exceptional Crop* (pp. 13–45). Elsevier. <https://doi.org/10.1016/b978-0-12-821715-3.00002-2>

- Wieser, H., Koehler, P., & Scherf, K. A. (2020b). The Two Faces of Wheat. In *Frontiers in Nutrition* (Vol. 7). Frontiers Media S.A. <https://doi.org/10.3389/fnut.2020.517313>
- Wieser, H., Koehler, P., & Scherf, K. A. (2023a). Chemistry of wheat gluten proteins: Qualitative composition. In *Cereal Chemistry* (Vol. 100, Issue 1, pp. 23–35). John Wiley and Sons Inc. <https://doi.org/10.1002/cche.10572>
- Wieser, H., Koehler, P., & Scherf, K. A. (2023b). Chemistry of wheat gluten proteins: Quantitative composition. In *Cereal Chemistry* (Vol. 100, Issue 1, pp. 36–55). John Wiley and Sons Inc. <https://doi.org/10.1002/cche.10553>
- Woychik, J. H., Bousdy Hp; J. A., & Dimler, D. R. J. (1961). Starch Gel Electrophoresis of Wheat Gluten Proteins With Concentrated Urea. In *ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS* (Vol. 94).
- Wrigley, C. , & Békés, F. , & B. W. (2006). Gliadin and glutenin: the unique balance of wheat quality (F. B. W. B. C. Wrigley, Ed.).
- Xynias, I. N., Mylonas, I., Korpetis, E. G., Ninou, E., Tsaballa, A., Avdikos, I. D., & Mavromatis, A. G. (2020). Durum wheat breeding in the Mediterranean region: Current status and future prospects. In *Agronomy* (Vol. 10, Issue 3). MDPI AG. <https://doi.org/10.3390/agronomy10030432>
- Zhang, P., He, Z., Zhang, Y., Xia, X., Liu, J., Yan, J., & Zhang, Y. (2007). Pan bread and Chinese white salted noodle qualities of Chinese winter wheat cultivars and their relationship with gluten protein fractions. *Cereal Chemistry*, 84(4), 370–378. <https://doi.org/10.1094/CCHEM-84-4-0370>
- Zhang, W., Chao, S., Manthey, F., Chicaiza, O., Brevis, J. C., Echenique, V., & Dubcovsky, J. (2008). QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat. *Theoretical and Applied Genetics*, 117(8), 1361–1377. <https://doi.org/10.1007/s00122-008-0869-1>
- Zhang, W., & Dubcovsky, J. (2008). Association between allelic variation at the Phytoene synthase 1 gene and yellow pigment content in the wheat grain. *Theoretical and Applied Genetics*, 116(5), 635–645. <https://doi.org/10.1007/s00122-007-0697-8>
- Zhang, X., Liu, D., Zhang, J., Jiang, W., Luo, G., Yang, W., Sun, J., Tong, Y., Cui, D., & Zhang, A. (2013). Novel insights into the composition, variation, organization, and expression of the low-molecular-weight glutenin subunit

- gene family in common wheat. *Journal of Experimental Botany*, 64(7), 2027–2040. <https://doi.org/10.1093/jxb/ert070>
- Zhao, J., Zheng, X., Qiao, L., Ge, C., Wu, B., Zhang, S., Qiao, L., Feng, Z., & Zheng, J. (2020). Effects of HMW-GSs on quality related traits in wheat (*Triticum aestivum* L.) under different water regimes. *PLoS ONE*, 15(8 August). <https://doi.org/10.1371/journal.pone.0237711>
- Zhou, Z., Zhang, Z., Mason, A. S., Chen, L., Liu, C., Qin, M., Li, W., Tian, B., Wu, Z., Lei, Z., & Hou, J. (2021). Quantitative traits loci mapping and molecular marker development for total glutenin and glutenin fraction contents in wheat. *BMC Plant Biology*, 21(1). <https://doi.org/10.1186/s12870-021-03221-0>
- Zhu, T., Wang, L., Rimbert, H., Rodriguez, J. C., Deal, K. R., De Oliveira, R., Choulet, F., Keeble-Gagnère, G., Tibbits, J., Rogers, J., Eversole, K., Appels, R., Gu, Y. Q., Mascher, M., Dvorak, J., & Luo, M. C. (2021). Optical maps refine the bread wheat *Triticum aestivum* cv. Chinese Spring genome assembly. *Plant Journal*, 107(1), 303–314. <https://doi.org/10.1111/tpj.15289>
- Zingale, S., Spina, A., Ingrao, C., Fallico, B., Timpanaro, G., Anastasi, U., & Guarnaccia, P. (2023). Factors Affecting the Nutritional, Health, and Technological Quality of Durum Wheat for Pasta-Making: A Systematic Literature Review. In *Plants* (Vol. 12, Issue 3). MDPI. <https://doi.org/10.3390/plants12030530>

Annex 1

LAB PROTOCOL: LMW-GS ANALYSIS BY SDS-PAGE ELECTROPHORESIS. Protocol is based on Sing et al (1991), with minor changes and is intended for extraction of glutenins from wheat seeds or flour and SDS-PAGE protein separation for LMW-GS identification. It also allows the identification of HMW-GS. When the protocol is used for glutenin quantification the extraction is performed from 20mg of flour, and only 8ul should be loaded in the acrylamide gel, these steps and other important steps for glutenins quantification are highlighted in red.

A. REAGENTS

A.1. REAGENTS FOR GLUTENINS EXTRACTION

Note: All reagents are purchased from SIGMA if possible. The purity of the water significantly affects the gel resolution.

Sol. 1 (1M Tris, pH 8.0)	
Trizma	12.11g
H ₂ O	80ml
Adjust pH to 8.0 with HCl (5 N). Make up to 100 ml with H ₂ O ₂ . Keep at 4°C	

Sol. 2 Fresh made	
Propanol-1, 50% (v/v). Fresh made	

Sol. 3	
Sol. 2	10ml
Sol. 1	1.6ml
H ₂ O	up to 20ml
Keep at 4°C	

Sol. 4 (2% w/v SDS, 40% w/v Glycerine, 0.02% w/v bromophenol blue in Tris pH:8)	
SDS	0.2g
Glycerine	4g
Bromophenol Blue	2mg
Sol. 1	0.8ml
H ₂ O	up to 10ml

1. Sol. 3 + 1% w/v dithiotreitol (DTT) Fresh made		
N. samples	Dithiotreitol (DTT)	Sol. 3
15	20 mg	up to 2ml
20	25 mg	up to 2.5ml
30	40 mg	up to 4ml
40	50 mg	up to 5ml
70	70 mg	up to 7ml

2. Sol. 3 + 1.4 % 4-vinylpyridine <i>Fresh made</i>		
N. samples	4-vinylpyridine	Sol. 3
15	28 μ l	up to 2ml
20	35 μ l	up to 2.5ml
30	56 μ l	up to 4ml
40	70 μ l	up to 5ml
70	98 μ l	up to 7ml

A.2. REAGENTS FOR SDS-PAGE ELECTROPHORESIS

Sol. 5 (Acrylamide 35 % (p/v))	
Acrylamide	175g
H ₂ O	up to 500ml
<i>Filter and keep at 4°C</i>	

Sol. 6 (Bis-acrylamide 2% (p/v))	
Bis-acrylamide	4g
H ₂ O	up to 200ml
<i>Filter and keep at 4°C</i>	

Sol. 7 (1M Tris, pH 8.8)	
Trizma	60.55g
H ₂ O	250ml
<i>Cool to 13°C</i>	
<i>Adjust pH to 8.8 with HCl (5 N). (Initial pH: 10.5)</i>	
<i>Make up to 500 ml with H₂O. Keep at 4°C</i>	

Sol. 8	
SDS 10% (w/v).	
Prepare 200ml	

Sol. 9 <i>Fresh made</i>	
Ammonium persulfate 1% w/v.	
Prepare fresh	

Sol. 10 (Electrophoresis Buffer)				
	1 litre	2 litre	3 litre	5 litre
Glycine	14.1g	28.2g	42.3g	70.5g
Trizma	3g	6g	9g	15g
SDS	1g	2g	3g	5g
H ₂ O	up to 1l	up to 2l	up to 3l	up to 5l

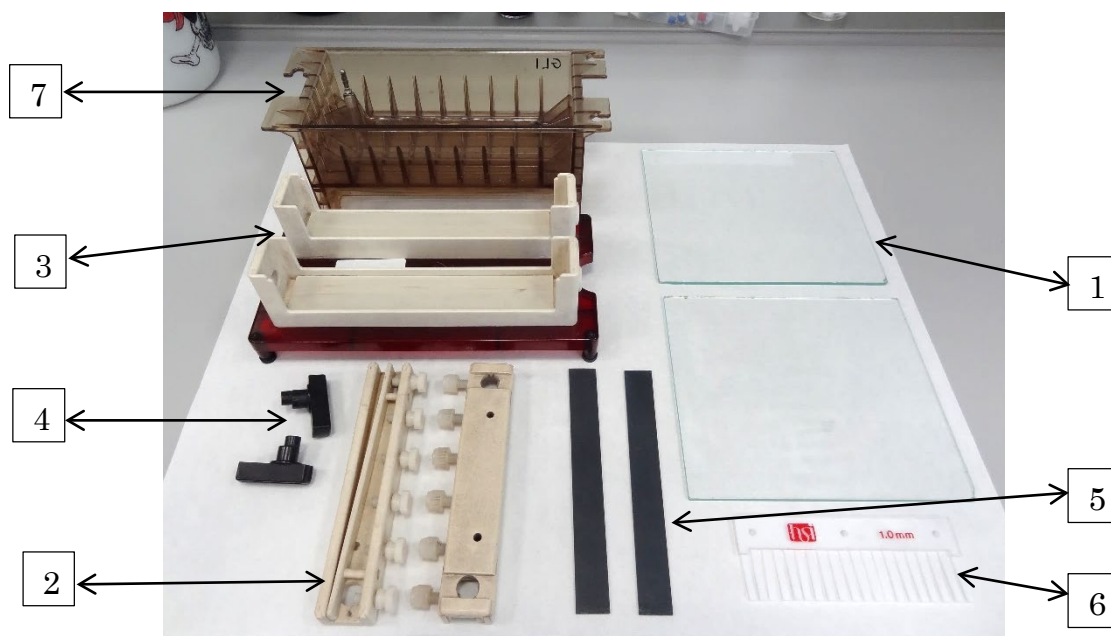
Sol. 11 (1M Tris, pH 6.8)	
Trizma	12.11g
H ₂ O	80ml
<i>Cool to 10°C. Adjust pH to 6.8 with HCl (35%) keeping cool. Make up to 100 ml with H₂O. Keep at 4°C</i>	

STAINING (Stock solution)	
Coomassie Brilliant Blue R-250	2g

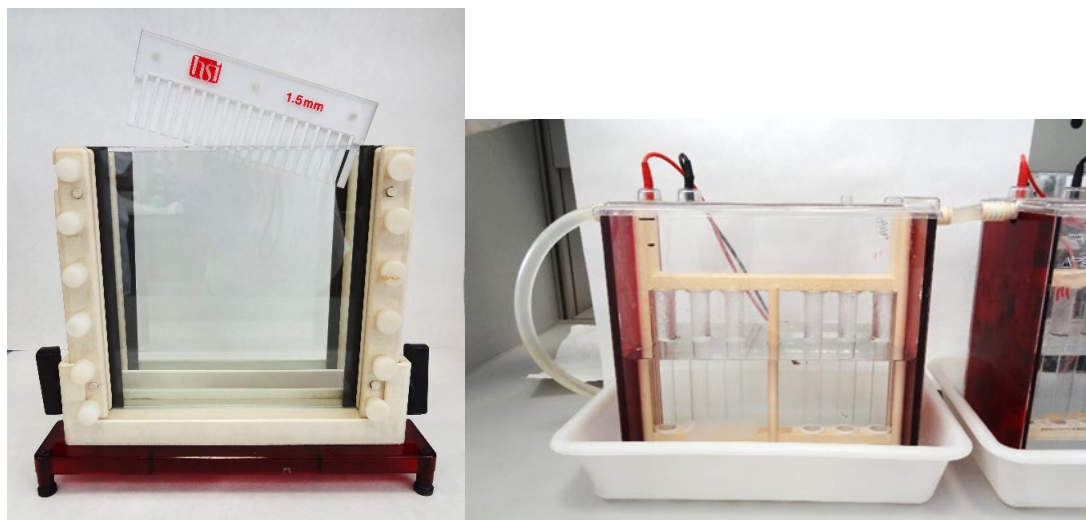
Ethanol 95%	500ml
<i>Leave o/n with agitation in the dark, and filter next day</i>	
STAINING Working Solution (fresh made, 250ml/gel)	
Stock solution	25ml
Trichloroacetic acid (TCA)	60g
H ₂ O	up to 500ml

B. EQUIPMENT

3. Vortex mixer
4. Heating oven or dry block heater
5. Bench centrifuge (capable of 13,000rpm)
6. Electrophoresis equipment. We use old Hoefer electrophoresis vertical units, the current models are Hoefer™ SE600X-15-1.5.
7. Cooling system for electrophoresis



Each gel will require two crystals (1), two lateral fasteners with screws to join them (2), a portage support (3), two black lateral screws (4), two plastic separators depending on the thickness of gel desired (1 mm or 1.5 mm) (5), a comb (15, 20 or 28 inserts) (6) and an upper adapter (7).



Follow manufacture's instructions for assembly.

C. PROCEDURE

C.1. GLUTENIN (HMW and LMW) EXTRACTION

The extraction is made from half grain of wheat finely ground in mortar or from **20 mg of flour** (for glutenins quantification always weight the sample). Samples are placed in 1.5 ml tubes.

Note: Work with gloves and gown, and under hood and mask when indicated by the protocol with.*

STEP 1. Gliadins washing

1. Add 1ml **Sol. 2**, vortex
2. Heat 30min at 65°C, vortex every 10min.
3. Centrifuge 5min / 13,000rpm
4. Discard the supernatant (contains gliadins)
5. Add 1ml **Sol. 2** to the residue and repeat steps 1-4
6. Wash the residue with 0.5 ml **Sol. 2** and repeat steps 1-4. Discard all the supernatant

STEP 2. Glutenins extraction

1. * Add 100µl **Sol. 3 + 1% dithiotreitol (DTT)** to the residue. (The DTT must be added at the last moment)
2. Vortex
3. Heat 30min at 65°C.
4. * Add 100µl **Sol. 3 + 1.4 % 4-vinylpyridine** (The vinylpyridine must be added at the last moment)
5. Vortex
6. Heat 15min at 65°C
7. Centrifuge 5min at 13,000rpm

8. Transfer 50 μ l to a new 1.5ml tube (the remaining sample can be keep at 4°C up to 3 days or freeze)
9. Add 50 μ l **Sol. 4**
10. Vortex.
11. Just before loading into the gel, heat 5min at 90°C with vigorous shaking (or heat 15min at 65°C) and centrifuge 5min / 13000rpm

C.2. LMW-GS ELECTROPHORESIS

Note: Work always with gloves and gown, and keep laboratory temperature below 26°C.

For LMW-Gs analysis, SDS-PAGE gels are made with 12% acrylamide solution in Tris-HCl pH:8.8. Large gels are recommended but shorts ones can also been made.

Large gels: 24.3 x 17.8 cm, 1mm thick.

Running conditions: At 40mA/gel for 5h or at 12.5mA/gel for 15h. 10°C cooling.

Short gels: 17 x 17.8 cm, 1mm thick.

Running conditions: At 40mA/gel for 3h30min or at 6.5 mA/gel for 17h. 10°C cooling.

When the blue dye comes out, the gel can be keep running for 10-20min more to obtain a better subunit separation.

For glutenins quantification use **Short gels:** 17 x 17.8 cm, 1mm thick.

Running conditions: At 40mA/gel for 3h30min. 10°C cooling. Stop running when the blue dye comes out.

GEL PREPARATION

Add sequentially in a glass baker with agitation all the components of SEPARATION GEL but Sol. 9 and TEMED, which are added at the very last moment.

SEPARATION GEL	For 2 short gels (1mm)
Sol. 5	17.5ml
Sol. 6	3ml
Sol. 7	18.8ml
H ₂ O	8.3ml
Sol. 8	0.5ml
Sol. 9	1.25ml
TEMED	25 μ l

Fill the space between the crystals with the gel by using a large syringe. It is recommended starting by a corner and doing it in a continuous and fast manner. Fill it until just below the level where the wells of the comb are going to reach, since that space will be filled with the stacking. The remaining gel solution from the beaker is allowed to polymerize and then removed with towel paper and throw it away to the general waste because it is no longer toxic.

Add a thin layer of butanol (about 2 mm) in order to achieve a homogeneous surface and let stand for polymerization. Once polymerized, remove butanol by pouring it and wash three times with distilled water.

Add sequentially in a glass baker with agitation all the components of STACKING GEL but Sol. 9 and TEMED, which will be added at the last moment.

STACKING GEL	Up to 4 gels
Sol. 5	1.71ml
Sol. 6	0.433ml
Sol. 11	2.5ml
H ₂ O	15.2ml
Sol. 8	0.2ml
Sol. 9	1ml
TEMED	20 μ l

Fill the space between the crystals with the stacking gel, by using a large syringe, until 1cm from the upper edge. Insert the comb and refill if necessary, being careful of not introduce bubbles. The remaining gel solution from the beaker is allowed to polymerize and then removed with towel paper and throw it away to the general waste because it is no longer toxic.

***For LMW-sS analysis it is recommended using 15 wells combs. For glutenins quantification 20 wells combs can be used.**

LOADING THE GEL

Once polymerized, remove the comb, pulling both ends at the same time. Electrophoresis buffer is added until all the air is eliminated.

The gels are numbered in the upper left corner, where the insertion of the samples begins. **The same standard sample should be inserted five times**, in the first and in the second well, as well as in the penultimate and last well, and in a middle one to check the gel horizontality. We recommend load **6 standards and 5 samples per gel** for glutenin alleles identification. Between 8 and 12 μ l of sample are loaded in each well. **For glutenins quantification only 8 μ l of sample are loaded, besides a well should be reserved for control sample to be load in all the gels in order to allow normalization.**

The gel number, the samples loaded on it and the date are recorded in the notebook.

Once the system is ready, it is introduced into the cuvette and filled up to $\frac{3}{4}$ parts with electrophoresis buffer. The upper adapter is filled with the same buffer until the beginning of the terminal (the platinum wire must always be covered). The lid is placed on top.

The system cooling is turned on at 10°C (check always that the tank is full of distilled water).

The wires are connected to the source and programmed for the desired mA and time.

STAINING THE GEL

Once the electrophoresis is finished, the gels are removed from the cuvette. The electrophoresis buffer of the upper adapter is emptied and the upper support of the crystals is removed.

To separate the crystals, first unscrew the side clips and remove them, and then remove the plastic spacers. Then, prying, separate the crystals.

We remove the stacking gel with a piece of paper and cut the lower left or right corner of the gel, to identify the first sample insertion.

The gel is carefully placed in a numbered box (note the box number in the notebook, next to the corresponding gel number and which corner has been cut) with 250 ml of staining solution for a small gel or 500 ml for a large gel and let stain o/n.

All parts of the system are washed and rinsed well. The crystals are scrubbed with a sponge that does not scratch, removing all traces of gel. Afterwards, they are rinsed with distilled water.

After 15h (approx), take out the gels and have a look at them in the white light illuminator. The proteins must be clearly seen, although sometimes more time in dye is required. In the case the proteins are well stained, for eliminating the blue background, discard the dye in the waste can (you can also reutilize this dye for gliadin staining) and replace it with water, rinsing a couple of times to eliminate dye remaining of the box. Let it fade in the water for a few hours or o/n. The expected result is shown in the Figure below. For glutenins quantification be sure gel is not stained for longer than 16 hours. Distain during 16 hours and take a gel image with a Molecular Imager® Gel Doc™ XR+ System (Bio-Rad, Hercules, California) under white light epi-illumination, optimizing the exposure time for faint bands, with ImageLab software v6.0.1 (Bio-Rad, Hercules, California).

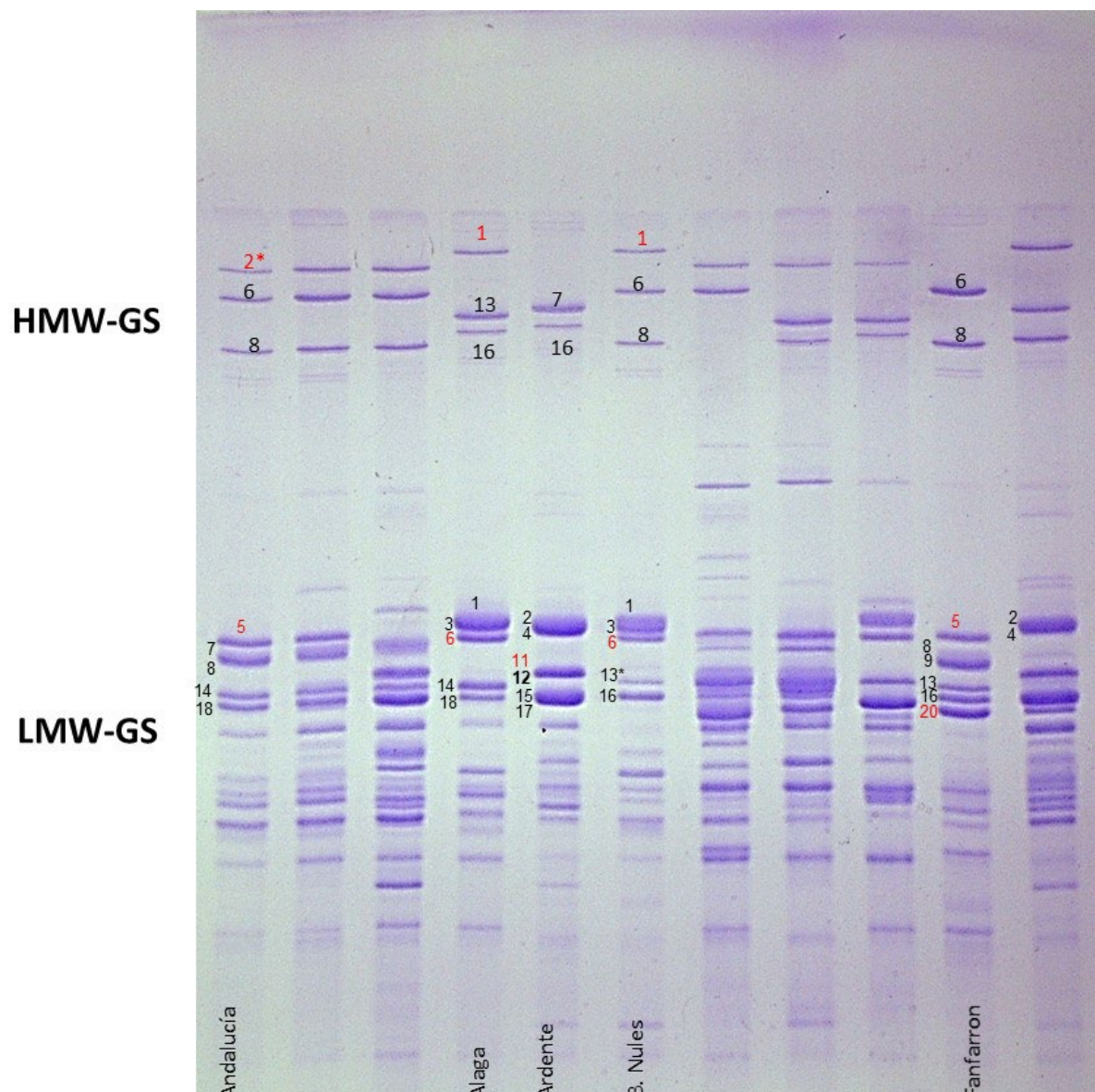
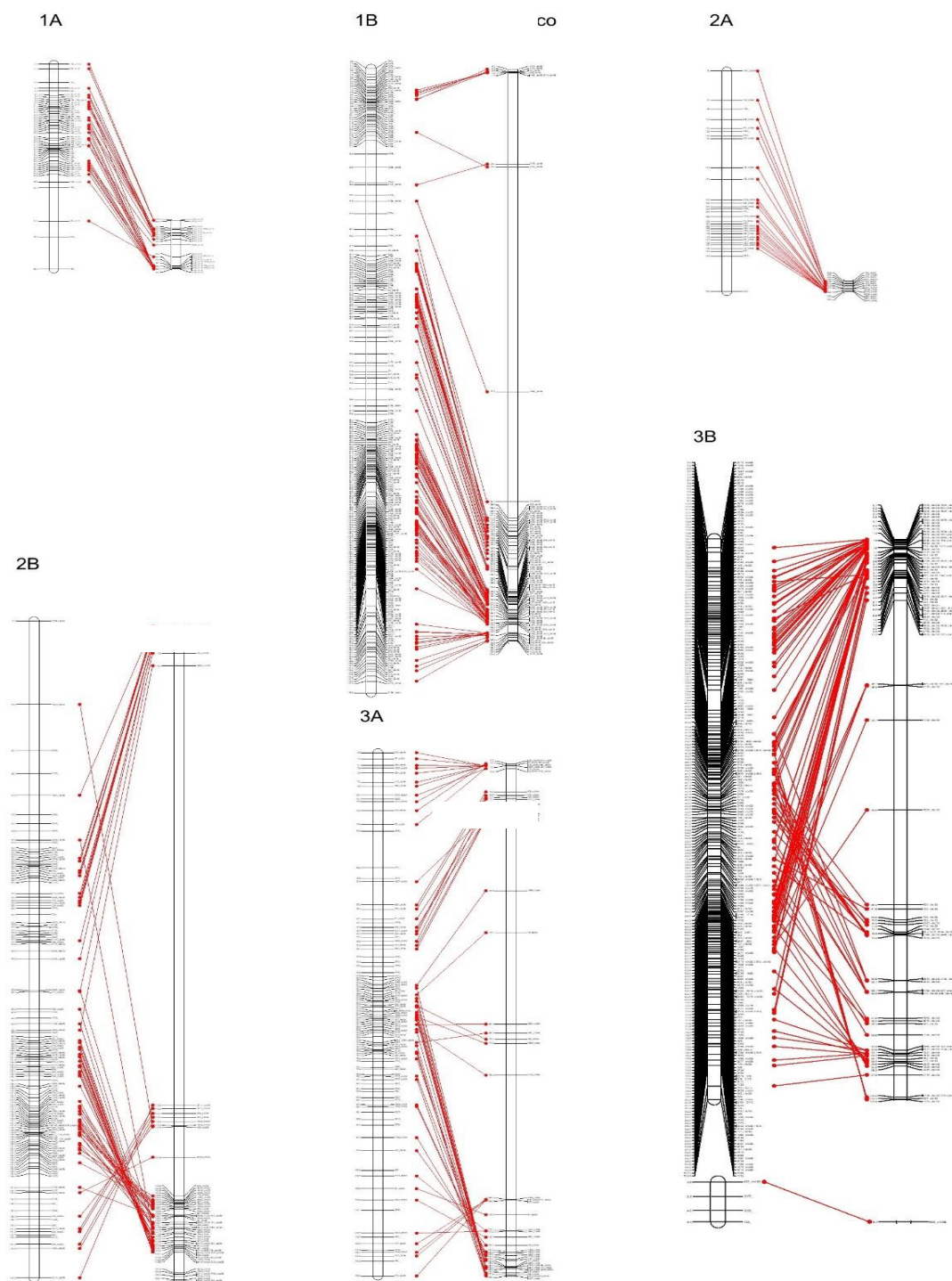


Figure. SDS-PAGE of High-molecular-weight (HMW) and Low-molecular weight (LMW) glutenin subunits of some durum wheat accessions and standard varieties. Some HMW and LMW glutenin subunits are indicated in red, black and bold black symbols for Glu-A1 and Glu-A3, Glu-B1 and Glu-B3, and Glu-B2 loci, respectively

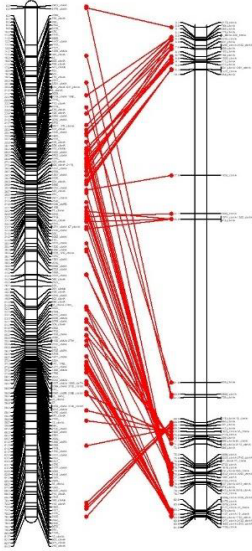
To keep the gels dried, two cellophane sheets, one "old" crystal and a 5% glycerol solution are required. The water is removed from the box with the gel, about 250 ml (or 500 ml for long gels) of 5% Glycerol is added to the gel and the two cellophane sheets are also placed there to hydrate. Let it stand for 15 minutes. On the glass, we point out the gel number, the box number and the date. Afterwards, place the first sheet of cellophane on the glass well stretched so that no bubbles remain. On top of this, place the gel. Wrap it all together with the second sheet, also taking care that no bubbles remain. Allow to dry until the gel is dehydrated (it can take a few days). Once dry, the cellophane sheets containing the gel are removed from the glass and the edges are cut around the gel. In that way, gels can be stored for a long time (years).

Annex 2

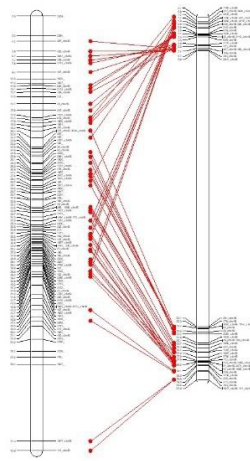
Comparison between the genetic map and the physical location in the reference genome.
Genetic map in the left and physic location in the right.



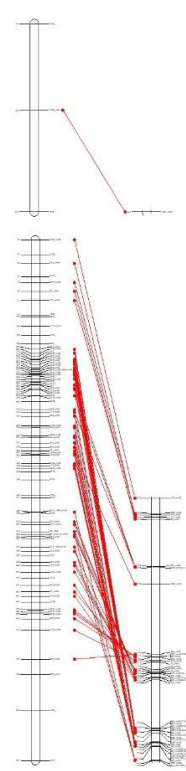
4A



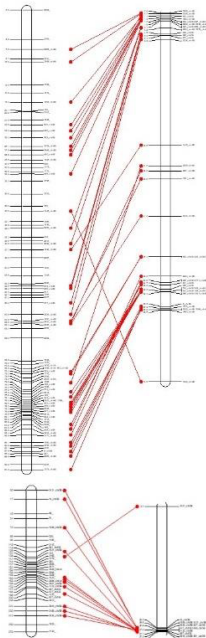
4B



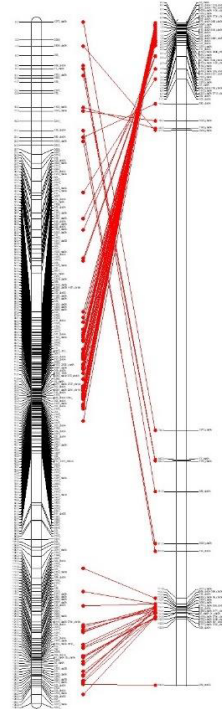
5A



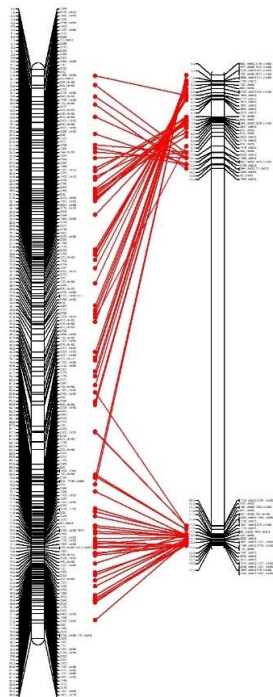
5B



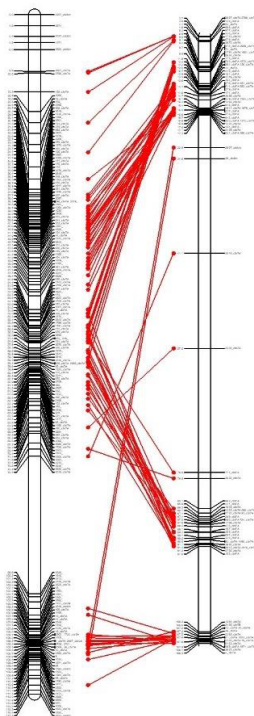
6A



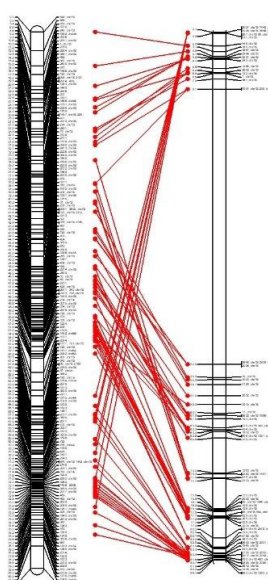
6B



7A



7B



Annex 3

Distribution of all QTL identified along the genetic map for TKW, GPC, flour color (b*) and grain size and shape traits (Ar, Perim, Majell, Minell). No blast indicates than it was not possible to locate the marker in the reference genome.

QTL	Chr	Start Position			Final Position			LOD Max	% Expl	variance	Seasons	Trait
		Position cM	Position pb	Marker name	Position cM	Position pb	Marker name					
b*_12/13_7A_1	7A	60.912	No Blast	4405981	60.912	No Blast	4405981	3.15	16.8%	0.641	2012_2013	b* valour of yellow color
b*_12/13_1B_1	1B	57.316	554316765	1261364	57.316	554316765	1261364	3.15	16.8%	0.641		
b*_13/14_7A_1	7A	104.718	No Blast	4405320	115.47	722949720	1669067	3.97	15.7%	0.379	2013_2014	
b*_19/20_7A_1	7A	91.146	No Blast	4407256	117.136	287614338	1082416	8.70	31.7%	0.460	2019_2020	
b*_20/21_7A_1	7A	86.146	275789455	1285300	117.136	287614338	1082416	8.69	31.4%	0.474	2020_2021	
Prot_12/13/1B_1	1B	66.643	581395374	2281267	67.643	No Blast	4406160	3.33	17.6%	2.060	2012_2013	Protein
Prot_12/13_4A_1	4A	60.681	79903905	1044739 F 0-26:G>A-26:G>A	60.681	79903905	1044739 F 0-26:G>A-26:G>A	4.20	12.7%	1.957		
Prot_13/14_____	/	/	/	/	LOD ≤3	/	/	/	/	/	2013-2014	
Prot_19/20_1B_1	1B	54.014	548453577	1082089	69.63	No Blast	4406160	5.57	21.7%	0.848	2019_2020	
Prot_20/21_1B_1	1B	22.601	740110752	1217640	30.542	No Blast	1087916	5.12	21.7%	0.848	2020_2021	
Prot_20/21_1B_2	1B	45.12	No Blast	3024514 F 0-9:G>A-9:G>A	57.891	No Blast	2331017	5.12	21.7%	0.848		
TKW_12/13_____	/	/	/	/	LOD ≤3	/	/	/	/	/	2012-2013	Thousand kernel weight
TKW_13/14_1B_1	1B	46.873	536206680	2278693	46.873	536206680	2278693	3.07	15.9%	5.966	2013_2014	
TKW_13/14_4B_1	4B	10.356	No Blast	1208119	11.618	37707737	2283875 F 0-40:C>T-40:C>T	4.01	12.4%	6.213		
TKW_19/20_____	/	/	/	/	LOD ≤3	/	/	/	/	/	2019_2020	
TKW_19/20	/	/	/	/	LOD ≤3	/	/	/	/	/	2020-2021	

Ar_12/13_____	/	/	/	/	LOD ≤3	/	/	/	/	/	2012-2013	AREA
Ar_13/14_4B_1	4B	8.944	51845399	1281577	14.661	35795268	985312 F 0-35:G>A-35:G>A	4.83	18.8%	0.470	2013_2014	
Ar19/20_4A_1	4A	21.039	No Blast	1089745	21.039	No Blast	1089745	4.84	18.8%	0.770	2019_2020	
Ar19/20_4A_2	4A	31.854	No Blast	3936040	39.773	631550286	978708 F 0-14:G>A-14:G>A	4.84	18.8%	0.770		
Ar19/20_4A_3	4A	60.405	686724985	1131505 F 0-16:C>A-16:C>A	64.675	No Blast	1279340 F 0-36:C>G-36:C>G	4.84	18.8%	0.770		
Ar19/20_4A_4	4A	72.484	No Blast	4407365	75.808	No Blast	100190526 F 0-17:T>C-17:T>C	4.84	18.8%	0.770		
Ar_19/20_4B_1	4B	16.837	1025888	1262162	21.42	8811144	1109756	3.24	13%	0.835		
Ar_19/20_4B_2	4B	28.669	No Blast	1092216 F 0-10:C>G-10:C>G	29.092	325902643	1111564	3.24	13%	0.835		
Ar_20/21_1B_1	1B	126.013	653506561	1366195	128.013	No Blast	1371494	3.70	17.7%	0.538	2020_2021	
Ar_20/21_2B_1	2B	69.15	No Blast	4993724 F 0-34:C>T-34:C>T	69.15	No Blast	4993724 F 0-34:C>T-34:C>T	3.03	12.2%	0.553		
Ar_20/21_4A_1	4A	37.839	No Blast	1091678 F 0-40:C>A-40:C>A	37.893	No Blast	1091678 F 0-40:C>A-40:C>A	3.02	12.2%	0.553		
Ar_20/21_4B_1	4B	5.878	61721903	1049581 F 0-33:T>C-33:T>C	29.61	No Blast	1139773	4.86	18.9%	0.511		
Perim_12/13_____	/	/	/	/	LOD ≤3	/	/	/	/	/	2012_2013	Perim
Perim_13/14_4A_1	4A	21.039	No Blast	1089745	21.039	No Blast	1089745	4.23	16.7%	0.003	2013_2014	
Perim_13/14_4A_2	4A	31.854	No Blast	3936040	37.893	No Blast	1091678 F 0-40:C>A-40:C>A	4.23	16.7%	0.003		

Perim_13/14_4B_1	4B	10.356	No Blast	2275559	13.538	No Blast	3953436 F 0-24:G>A-24:G>A	4.16	16.4%	0.0032		Major ellipse
Perim_13/14_4B_2	4B	28.669	No Blast	1092216 F 0-10:C>G-10:C>G	29.092	325902643	1111564	416	16.4%	0.0032		
Perim_13/14_6B_1	6B	44.232	16077801	4004505 F 0-13:A>G-13:A>G	44.232	16077801	4004505 F 0-13:A>G-13:A>G	3.11	12.5%	0.0034		
Perim_19/20_3B_1_1	3B_1	86.125	80952893	1107538	86.125	80952893	1107538	3.05	13.3%	0.0051	2019_2020	
Perim_19/20_4A_1	4A	21.039	No Blast	1089745	21.039	No Blast	1089745	3.91	15.5%	0.004		
Perim_19/20_4A_2	4A	37.839	No Blast	1091678 F 0-40:C>A-40:C>A	39.773	631550286	978708 F 0-14:G>A-14:G>A	3.91	15.5%	0.004		
Perim_19/20_4A_3	4A	75.085	No Blast	3934106	75.808	No Blast	100190526 F 0-17:T>C-17:T>C	3.91	15.5%	0.004		
Perim_19/20_6B_1	6B	31.59	No Blast	32940644	36.246	22314036	1205396 F 0-36:G>A-36:G>A	3.26	13.1%	0.0051		
Perim_19/20_6B_2	6B	44.232	16077801	4004505 F 0-13:A>G-13:A>G	44.569	16221973	4009312 F 0-37:T>G-37:T>G	3.26	13.1%	0.0051		
Perim_20/21_4A_1	4A	21.039	No Blast	1089745	21.039	No Blast	1089745	4.49	17.6%	0.002		
Perim_20/21_4A_2	4A	31.854	No Blast	3936040	31.854	No Blast	3936040	4.49	17.6%	0.002		
Perim_20/21_4A_3	4A	36.839	324323069	1220225 F 0-34:C>A-34:C>A	37.893	No Blast	1091678 F 0-40:C>A-40:C>A	4.49	17.6%	0.002		
Perim_20/21_4B_1	4B	11.306	No Blast	5412102	29.61	No Blast	1139773	5.80	22.1%	0.0026	2012_2013	
Majell_12/13	/	/	/	/	LOD ≤3	/	/	/	/	/		
Majell_13/14_3B_1_1	3B_1	86.125	80952893	1107538	86.125	80952893	1107538	3.25	13%	0.038		
Majell_13/14_4A_1	4A	21.039	No Blast	1089745	21.039	No Blast	1089745	5.09	19.7%	0.035		

Majell_13/14_4A_2	4A	30.922	624511003	1373354	39.773	631550286	978708 F 0-14:G>A-14:G>A	5.09	19.7%	0.035		
Majell_13/14_4B_1	4B	10.356	No Blast	1208119	11.618	37707737	2283875 F 0-40:C>T-40:C>T	3.92	15.5%	0.037		
Majell_13/14_4B_2	4B	28.669	No Blast	1092216 F 0-10:C>G-10:C>G	29.092	325902643	1111564	3.92	15.5%	0.037		
Majell_19/20_3B_1_1	3B_1	86.125	80952893	1107538	86.125	80952893	1107538	3.06	12.3%	0.065	2019_2020	
Majell_19/20_4A_1	4A	21.039	No Blast	1089745	21.039	No Blast	1089745	3.88	15.4%	0.063		
Majell_19/20_4A_2	4A	37.839	No Blast	1091678 F 0-40:C>A-40:C>A	39.773	631550286	978708 F 0-14:G>A-14:G>A	3.88	15.4%	0.063		
Majell_19/20_4A_3	4A	60.405	686724985	1131505 F 0-16:C>A-16:C>A	60.628	No Blast	1123787	3.88	15.4%	0.063		
Majell_19/20_4A_4	4A	75.696	No Blast	3934106	75.808	No Blast	100190526 F 0-17:T>C-17:T>C	3.88	15.4%	0.063	2019_2020	
Majell_20/21_3A_1	3A	53.865	No Blast	1208039	53.865	No Blast	1208039	3.37	13.5%	0.038	2020_2021	
Majell_20/21_3A_2	3A	61.209	No Blast	4009543	61.209	No Blast	4009543	3.37	13.5%	0.038		
Majell_20/21_4A_1	4A	10.044	No Blast	4404781	10.044	No Blast	4404781	4.82	18.7%	0.035		
Majell_20/21_4A_2	4A	21.039	No Blast	1089745	21.039	No Blast	1089745	4.82	18.7%	0.035		
Majell_20/21_4A_3	4A	36.839	324323069	1220225 F 0-34:C>A-34:C>A	37.893	No Blast	1091678 F 0-40:C>A-40:C>A	4.82	18.7%	0.035		
Majell_20/21_4B_1	4B	16.837	31208248	100175862 F 0-32:A>G-32:A>G	17.013	1025888	1262162	5.19	20%	0.035		
Majell_20/21_4B_2	4B	23.343	No Blast	978721 F 0-18:C>T-18:C>T	29.61	No Blast	1139773	5.19	20%	0.035		

Minell_12/13_5B_1	5B_2	16.769	690582738	1052428	23.264	684243983	1278683	3.10	16.5%	0.009	2012_2013	Minor ellipse
Minell_12/13_6B_1	6B	56.607	No Blast	3533124	58.296	No Blast	1229701	3.94	20.5%	0.008		
Minell13/14_1B_1	1B	46.873	536206680	2278693	46.873	536206680	2278693	3.01	12.2%	0.005	2013_2014	
Minell_13/14_4B_1	4B	11.618	37707737	2283875 F 0-40:C>T-40:C>T	11.618	37707737	2283875 F 0-40:C>T-40:C>T	3.45	13.8%	0.0051		
Minell_19/20_4A_1	4A	57.219	No Blast	1719781	57.219	No Blast	1719781	3.69	14.7%	0.0068	2019_2020	
Minell_19/20_4A_2	4A	75.085	No Blast	3934106	75.808	No Blast	100190526 F 0-17:T>C-17:T>C	3.69	14.7%	0.0068		
Minell_19/20_4B_1	4B	9.356	54855265	100143952 F 0-42:T>G-42:T>G	14.661	35795268	985312 F 0-35:G>A-35:G>A	3.30	13.3%	0.0069		
Minell_19/20_7B_1	7B	28.642	453440752	1099093 F 0-38:G>A-38:G>A	28.642	453440752	1099093 F 0-38:G>A-38:G>A	3.62	14.4%	0.0068		
Minell_12/20_7B_2	7B	45.478	521973539	1137091 F 0-48:A>C-48:A>C	45.478	521973539	1137091 F 0-48:A>C-48:A>C	3.62	14.4%	0.0068		
Minell_20/21_1B_1	1B	34.881	No Blast	2304066	34.881	No Blast	2304066	3.12	12.6%	0.0059	2020_2021	
Minell_20/21_4B_1	4B	5.878	61721903	1049581 F 0-33:T>C-33:T>C	6.68	63742193	1281664	3.79	15.1%	0.0057		
Minell_20/21_4B_2	4B	12.667	59242413	23884414 F 0-33:G>A-33:G>A	12.667	59242413	23884414 F 0-33:G>A-33:G>A	3.79	15.1%	0.0057		

Annex 4

Distribution of all QTL identified along the genetic map for S1, S2, S3, S5, HMW-GS and LMW-GS. The traits were normally distributed, so the analysis was performed with SIM. No blast indicates than it was not possible to locate the marker in the reference genome.

QTL	Chr	Position Star			Final position			LOD	%			
		Position cM	Position pb	Marker name	Position cM	Position pb	Marker name	Max	Expl	variance	Years	Trait
S1_19/20_1B_1	1B	13.394	No blast	1242215	13.394	No blast	1242215	3.16	12.8%	3.87308e ⁻⁰⁷	2019_2020	S1
S1_19/20_6A_1	6A	130.125	61384353 5	1233246	131.625	No blast	2276059	3.54	14.2%	3.81088e ⁻⁰⁷		
S1_20/21_1B_1	1B	1	No blast	1102121	15.164	No blast	1259845	4.46	17.6%	1.95446e ⁻⁰⁷	2020_2021	
S1_20/21_7A_1	7A	44.275	No blast	1390825	44.275	No blast	1390825	3.59	14.4%	2.03018e ⁻⁰⁷		
S2/_____	/	/	/	/	LOD ≤3	/	/	/	/	/	2019_2020	S2
S2_20/21_4A_1	4A	54.698	90521147	1378716 F 0- 16:C>T- 16:C>T	65.155	No blast	4911183 F 0- 10:G>C-10:G>C	4.49	17.7%	1.82279e ⁻⁰⁷	2020_2021	
S2_20/21_7A_3	7A	29.334	No blast	3946449	29.334	No blast	3946449	3.31	13.4%	1.91890e ⁻⁰⁷		
S3_19/20_1B_1	1B	2.053	No blast	3022546	13.394	No blast	1242215	4.89	19.2%	64.3388	2019_2020	S3
S3_20/21_1B_1	1B	2.053	No blast	3022546	16.142	No blast	2303647	4.75	19.2%	64.3388	2020_2021	
S5_19/20_1B_1	1B	1	No blast	1102121	17.236	No blast	3948292	7.07	26.5%	55.2782	2019_2020	S5

S5_20/21_1B_1	1B	0	No blast	1122863	18.236	No blast	3948292	10.0 0	35.2%	25.3324	2020_2021	
S5_20/21_7A_1	7A	24.722	31142682	1187043 F 0- 31:A>T- 31:A>T	54.614	654336215	23884475 F 0- 16:T>C-16:T>C	4.62	18.2%	31.9990		
HMW_19/20_1B_1	1B	0	No blast	1122863	17.142	No blast	3948292	5.23	20.5%	0.0144880	2019_2020	HMW
HMW_20/21____	/	/	/	/	LOD ≤ 3	/	/	/	/	/	2020_2021	
LMW_19/20_1B_1	1B	0	No blast	1122863	17.236	No blast	3948292	8.01	29.6%	0.00979	2019_2020	LMW
LMW_19/20_1B_2	1B	42.277	No blast	3222160 F 0- 21:T>A- 21:T>A	45.12	No blast	3024514 F 0- 9:G>A-9:G>A	8.01	29.6%	0.00979		
LMW_19/20_1B_3	1B	51.787	No blast	1091605 F 0- 41:A>G- 41:A>G	64.054	No blast	3021884	8.01	29.6%	0.00979		
LMW_20/21____	/	/	/	/	LOD ≤ 3	/	/	/	/	/	2020_2021	

Annex 5

Distribution of all QTL identified along the genetic map for SDSs, S4, HMW-GS to LMW-GS ratio. The traits were not normally distributed, so the analysis was performed with Klustall-Wallis. No blast indicates than it was not possible to locate the marker in the reference genome.

QTL	Chr	Position Start			Final position			K* Max	Seasons	Trait
		Position cM	Position pb	Marker name	Position cM	Position pb	Marker name			
SDS_19/20_1B_1	1B	0	No blast	1122863	17.236	No blast	3948292	46.267	2019_2020	SDSS
SDS_19/20_1B_2	1B	34.881	No blast	2304066	70.661	59110526	1062692	46.267		
SDS_19/20_6B_1	6B	38.671	17996686	2260491	38.671	17996686	2260491	9.841		
SDS_19/20_6B_2	6B	49.802	44464188	1022880	49.802	44464188	1022880	9.841		
SDS_20/21_1B_1	1B	0	No blast	1122863	17.236	No blast	3948292	52.221	2020_2021	
SDS_20/21_1B_2	1B	31.921	383646486	1255054	73.552	589596941	1096609	52.221		
SDS_20/21_3B_1	3B	89.03	No blast	1089859	89.03	No blast	1089859	8.17		
SDS_20/21_6B_1	6B	38.671	17996686	2260491	39.128	No blast	3960546	9.777		
SDS_20/21_6B_2	6B	49.802	44464188	1022880	49.802	44464188	1022880	9.777		
S4_19/20_3A_1	3A	123.327	638061021	1218916 F 0-58:G>A-58:G>A	124.609	No blast	100185750 F 0-28:A>G-28:A>G	17.001	2019_2020	S4
S4_19/20_6A_1	6A	80.509	No blast	2291983	80.509	No blast	2291983	8.084		
S4_19/20_7A_1	7A	55.785	590065187	1269396	55.785	590065187	1269396	8.084		

S4_20/21_4A_1	4A	43.828	No blast	3020472 F 0-13:C>T-13:C>T	43.828	No blast	3020472 F 0-13:C>T-13:C>T	18.862	2020_2021	Ratio HMW/LMW
S4_20/21_7A_1	7A	53.163	85270676	1274875 F 0-50:G>A-50:G>A	53.163	85270676	1274875 F 0-50:G>A-50:G>A	12.511		
RATIO_19/20_7B_1	7B	10.778	54788102	1143330 F 0-49:T>C-49:T>C	10.778	54788102	1143330 F 0-49:T>C-49:T>C	12.457	2019_2020	
RATIO_19/20_7B_2	7B	51.167	637083477	2279311 F 0-44:T>C-44:T>C	51.167	637083477	2279311 F 0-44:T>C-44:T>C	12.457		
RATIO_19/20_3A_1	3A	56.685	No blast	5580220	56.685	No blast	5580220	9.661		
RATIO_20/21_3A_1	3A	61.209	No blast	4009543	64.72	414341416	1719863	10.983	2020_2021	
RATIO_20/21_3B_1	3B	6.033	No blast	4404644	6.033	No blast	4404644	10.428		
RATIO_20/21_3B_2	3B	26.684	No blast	1090077	26.684	No blast	1090077	10.428		
RATIO_20/21_4A_1	4A	53.163	No blast	3020472 F 0-13:C>T-13:C>T	53.163	No blast	3020472 F 0-13:C>T-13:C>T	18.872		
RATIO_20/21_4B_1	4B	43.296	613209062	1100014 F 0-24:T>C-24:T>C	43.296	613209062	1100014 F 0-24:T>C-24:T>C	12.559		
RATIO_20/21_4B_2	4B	56.187	No blast	4009440	57.186	No blast	3025431 F 0-20:A>C-20:A>C	12.559		
RATIO_20/21_5B_1	5B	90.5	No blast	3952912	90.5	No blast	3952912	10.076		
RATIO_20/21_5B_2	5B	24.247	681522371	1404273	24.247	681522371	1404273	8.744		
RATIO_20/21_6A_1	6A	83.311	No blast	3384758	83.311	No blast	3384758	8.832		
RATIO_20/21_7B_1	7B	25.378	579562653	1127482	25.378	579562653	1127482	13.025		
RATIO_20/21_7B_2	7B	40.026	No blast	3936564	50.849	No blast	100125078 F 0-10:C>T-10:C>T	13.025		