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Molecular characterization of candidate genes involved in nitrogen metabolism and relationship with the grain protein content of wheat

Agata Gadaleta, Domenica Nigro, Ilaria Marcotuli, Angelica Giancaspro, Antonio Blanco

Department of Soil, Plant and Food Sciences, Section of Genetics and Plant Breeding, University of Bari "Aldo Moro", Bari, Italy

Abstract. Wheat is one of the most important cereal crops grown worldwide and provides most of the proteins in human diet. Grain protein content (GPC) determines the nutritional value and the baking properties of common wheat (*Triticum aestivum* L. *ssp. aestivum*) as well as the pasta-making technology characteristics of durum wheat (*Triticum turgidum* L. *ssp*. *durum*). GPC is a typical quantitative trait controlled by a complex genetic system and influenced by environmental factors and management practices, as well as nitrogen and water availability, temperature and light intensity. In higher plants inorganic nitrogen, in the form of ammonia, is assimilated via the glutamate synthase cycle or GS-GOGAT pathway. This assimilation requires cofactors, reducing equivalents and carbon skeletons generated during photosynthesis. We focused on the Glutamine synthetase and Glutamate synthase, as potential candidates for determining grain protein content. While Glutamine synthetase genes are a family whose enzymes are located in both cytoplasm and plastids, glutamate synthase exists in two different isoform depending on the electron donor used as cofactor, NADH-dependent and Fd- dependent GOGAT, both active in plastids. In the present manuscript has been reported an overview on the candidate gene involved found in the control of grain protein content.

Keywords. Wheat – Glutamine synthetase (GS) – Glutamate synthase (GOGAT) – Sequencing – Real Time PCR.

Caractérisation moléculaire des gènes candidats impliqués dans le métabolisme de l'azote et relation avec la teneur en protéines du grain de blé

Résumé. *Le blé est l'une des cultures céréalières les plus importantes dans le monde entier et il fournit la plupart des protéines de l'alimentation humaine. La teneur en protéines des grains (GPC) détermine la valeur nutritionnelle et les propriétés de cuisson du blé commun (Triticum aestivum L. ssp. aestivum) ainsi que les caractéristiques de la production de pâtes du blé dur (Triticum turgidum L. ssp. durum). La GPC est un caractère quantitatif typique contrôlé par un système génétique complexe et influencé par des facteurs environnementaux et les pratiques de gestion, ainsi que par la disponibilité de l'azote et de l'eau, la température et l'intensité lumineuse. Chez les plantes supérieures, l'azote inorganique, sous forme d'ammoniac, est assimilé par l'intermédiaire du cycle de la glutamate synthase ou la voie GS-GOGAT. Cette assimilation nécessite des cofacteurs, des équivalents réducteurs et des squelettes de carbone, générés lors de la photosynthèse. Nous nous sommes concentrés sur la glutamine synthétase et la glutamate synthase, comme des candidats potentiels pour déterminer la teneur en protéines du grain. Alors que les gènes de la glutamine synthétase sont une famille dont les enzymes sont situés à la fois dans le cytoplasme et les plastes, la glutamate synthase existe en deux isoformes différents selon le donneur d'électrons utilisé comme cofacteur, NADH dépendant et GOGAT Fd- dépendant, tous les deux actifs dans les plastes. Dans le présent travail, nous allons présenter une vue d'ensemble des gènes candidats impliqués dans le contrôle de la teneur en protéines du grain.*

Mots-clés. *Blé – Glutamine synthétase (GS) – Glutamate synthase (GOGAT) – Séquençage – PCR en temps réel.*

I – Introduction

The nutritional quality of cereals is an important component of the population diet as the cereals represent the largest component of world food supplies. In the years ahead wheat, perhaps more than other cereals, can be expected to assume greater importance as a source of protein for much of the world's increasing population. In durum wheat, seed storage proteins are important not only from the nutritional standpoint, but they have even greater significance for pasta-making quality. Grain protein concentration, protein quality, are same of the major quality attributes affecting pasta-making technology characteristics and resistance to overcooking (Autran *et al*., 1996). The accumulation of protein in kernels is related to the nitrogen availability. Nitrogen uptake is an essential element in crop improvement, either directly for grain protein content or indirectly for photosynthetic production. Thus, nitrogen utilization is fundamental to crop productivity, and over the past 50 years nitrogen (N) fertilizers have been extensively used to increase both grain yield (GY) and grain protein content (GPC) in cereals and wheat - helping to support a vastly increased world population. However, this requires that growers must optimize the use of N fertilizers to avoid pollution, while maintaining reasonable profit margins. Such crops would make better use of nitrogen fertilizer supplies giving higher yields with improved protein contents. Therefore, selecting new crop varieties exhibiting improved nitrogen use efficiency (NUE; the yield of grain per unit of available N in the soil), and adapting agricultural practices to reduce the use of N fertilizers both represent challenges for both breeders and farmers (Hirel, 2007).

Whether N is derived from soil reserves, from N fertilizer, or from N_2 fixation, it is incorporated into the organic form via the assimilation of ammonia. However, the primary assimilation of ammonia from external inorganic N is only part of the process. N is also released from organic combination as ammonia and re-assimilated many times during the movement of N around the plant, from seed reserve, through transport to vegetative organs, to eventual re-deposition in a new crop of seeds. There is also a major release and re-assimilation of N during the process of photorespiration in C3 plants. The process of ammonia assimilation is thus of crucial importance to crop growth and productivity.

II – Detection of QTL for grain protein content

GPC is a typical quantitative trait controlled by a complex genetic system and influenced by environmental factors and management practices (nitrogen and water availability, temperature and light intensity). Heritability estimates for GPC ranged from 0.41 (Kramer 1979) to 0.70 (Suprayogi *et al*., 2009), depending upon the genetic material, environment and the method of computation. The extensive review by Konzak (1977) and more recent investigations by (Levy and Feldman 1989; Stein *et al*., 1992; Snape *et al*., 1995; Sourdille *et al*., 1996; Joppa *et al*., 1997; Prasad *et al*., 1999; Khan *et al*., 2000; Perretant *et al*., 2000; Dholakia *et al*., 2001; Zanetti *et al*., 2001; Campbell *et al*., 2001; Börner *et al*., 2002; Blanco *et al*. 2002, 2006; Olmos *et al*. 2003; Groos *et al*., 2003; Prasad *et al*., 2003; Gonzales-Hernandez *et al*., 2004; Turner *et al*., 2004; Huang *et al*., 2006; Nelson *et al*., 2006; Zhang *et al*., 2008; Mann *et al*., 2009; Raman *et al*., 2009; Suprayogi *et al*., 2009; Sun *et al*., 2010) have indicated that factors influencing protein concentration in cultivated and wild wheats are located on all chromosomes. The lack of sufficient genetic variation for useful traits within the cultivated wheats has limited the ability of plant breeders to improve grain yield and grain quality.

Recently Blanco *et a*l. (2012) in their study evaluated grain yield components and GPC in five field trials with twelve replicates and in a RIL population derived by the cross of two durum wheat cultivars Ciccio x Svevo. Ten independent genomic regions involved in the expression of GPC were identified, six of which were associated with QTLs for one or more grain yield components. QTL alleles with increased GPC effects were associated with QTL alleles with decreased effects

on one or more yield component traits. Four QTLs for GPC showing always significant effects should represent genes that influence GPC independently from variation in the yield components. We compared the genomic regions involved in the quantitative expression of GPC found in the Svevo x Ciccio RIL population with the map position of QTLs found in different genetic materials. A major QTL on chromosome 2A was further investigated. The influence of group-2 chromosomes on GPC control was firstly reported by Joppa and Cantrell (1990) using durum wheat - var. *dicoccoides* chromosome substitution lines then confirmed by Blanco *et al*. (2006) in the durum backcross line 3BIL-85 (Latino x *dicoccoides*) and by Suprayogi *et al*. (2009) in the Canadian durum line DT695.

III – Candidate genes approach

The candidate gene approach has been applied in plant genetics in the past decade for the characterization and cloning of Mendelian and quantitative trait loci (QTLs) as a complementary strategy to map-based cloning and insertional mutagenesis. Candidate genes analysis is based on the hypothesis that known-function genes (the candidate genes, CGs) could correspond to loci controlling traits of interest (Gebhardt *et al*., 2007). CGs refer either to cloned genes presumed to affect a given trait ('functional CGs') or to genes suggested by their close proximity on linkage maps to loci controlling the trait ('positional CGs'). In plant genetics the most common way to identify a CG is to look for map co-segregation between CGs and loci affecting the trait. Statistical association analyses between molecular polymorphisms of the CG and variation in the trait of interest can let to affirm the involvement of the CG in a specific metabolic pathway. To select the most promising candidates from a large number of functional candidate genes, gene sequences should be tested for linkage to QTL for the trait of interest by molecular mapping, thereby identifying positional candidates (genes co-localizing with a QTL) (Pajerowska *et al*., 2005).

In the present work this approach has been applied to the study of grain protein content in durum wheat. Several studies have attested the key-role of the glutamine synthetase enzyme (GS) in plant nitrogen metabolism (Bernard *et al*., 2009) and GOGAT (glutamine-2-oxoglutarate amidotransferase). Glutamine synthetase gene encodes for an enzyme responsible of the first step of ammonium assimilation and transformation into glutamine and glutamate, essential compounds in aminoacid-biosynthetic pathway. GS exists in multiple enzyme forms with the chloroplastic isozyme encoded by one gene (GS2) and the cytosolic encoded by 3–5 genes depending on the species. Studies have shown that both GS isozymes are regulated in a developmental manner in leaves and have different metabolic roles (Tobin *et al*. 1985; Kamachi *et al*., 1991; Finnemann and Schjoerring 2000; Habash *et al*., 2001). Cytosolic GS has multiple metabolic functions, such as assimilating ammonia into glutamine for transport and distribution throughout the plant; immunolocalisation studies in tobacco (Brugie're *et al*., 1999), pine (Canovas *et al*., 2007), potato (Pereira *et al*., 1995) and rice (Sakurai *et al*., 1996; Tabuchi *et al*., 2005) have shown predominant vascular location in different organs. Whilst studies on GS regulation in several species have shown some common regulatory mechanisms, also highlighted differences particularly in gene expression, protein and enzyme activity levels (McNally *et al*., 1983). Few studies are available about genomic variation of these genes, therefore, it is important to study the role of each GS gene in a variety of plant species.

On the bases of phylogenetic studies and mapping data in wheat, ten *GS* cDNA sequences were classified into four sub-families denominate *GS1* (a, b, and c), *GS2* (a, b, and c), *GSr* (1 and 2) and *GSe* (1 and 2) (Bernard *et al*., 2008). Bernard *et al*. (2008) reported that QTLs for flag leaf total GS activity were positively co-localised with others for grain and stem nitrogen, but smaller correlations were established with loci for grain yield components; they identified QTLs for GS activity co-localised to a *GS2* gene mapped on chromosome 2A and to the *GSr* gene on 4A. Genetic studies in rice (Yamaya *et al*., 2002; Obara *et al*., 2004) and maize (Hirel *et al*., 2001,

2007; Galais and Hirel, 2004) demonstrated co-localisations of QTLs for GS protein or activity with QTLs relating to grain parameters at the mapped *GS* genes. Bernard *et al*. (2008) auspicated to integrate the biochemical and genetic approaches to further establish allelic differences in GS isozymes and to uncover new regulatory loci modulating GS activity in diverse genetic material or mapping populations. The GOGAT enzyme catalyzes the reductive transfer of the amide group of glutamine to 2-oxoglutarate to form two glutamate molecules (Krapp *et al*., 2005). Together with GS, it maintains the flow of N from NH_4^+ into glutamine and glutamate, which are then used for several other aminotransferase reactions during the synthesis of amino acids (Ireland and Lea, 1999). Kinetic and inhibitory studies have suggested that GOGAT is the rate-limiting step in amino acid production (Chen and Cullimore, 1989; Baron *et al*., 1994). In rice, two different GOGAT enzymes have been identified based on the electron donor: a ferredoxin (Fd)-dependent GOGAT and a NADH-dependent GOGAT. In rice, NADH-GOGAT is active in developing organs, such as unexpanded non-green leaves and developing grains (Yamaya *et al*., 2002). NADH-GOGAT has been proposed to be involved in the use of remobilized nitrogen, because it is located in the specific cell types which are important for solute transport from the phloem and xylem elements (Hayakawa *et al*., 1994).

In the present work we focused the attention on *GS* genes and GOGAT with the objectives to isolate and characterize the complete genomic sequences of these genes in the A and B genomes of two elite durum wheat cultivars differing for grain protein content and to assess the association with QTLs for grain protein content.

IV – GS-GOGAT candidate genes

The isolation of the complete glutamine synthetase gene sequences and the localization on the two homeologous chromosome 2A and 2B in the durum wheat cvs. Ciccio and Svevo characterized by different grain protein content has been reported by Gadaleta *et al*. (2011). *GS2-A2* located on 2A chromosome was found comprised of 13 exons separated by 12 introns The *GS2-B2* has the same intron/exon structure, but the two cultivars differ for the insertion of a 33 bp sequence located in the second intron of the cv. Svevo. The complete cytosolic glutamine synthetase gene sequences of the durum wheat cvs “Ciccio” and “Svevo” was also reported by Gadaleta *et al*. (2014). *GSe-A4* was found located on 4A chromosome and was comprised of 12 exons separated by 11 introns, while the *GSe-B4* gene on 4B chromosome was comprised of 11 exons separated by 10 introns (Gadaleta *et al*., 2014).

Specific primer were designed in the polymorphic regions and in order to genetically map the genes in a RIL population, obtained by crossing the two durum wheat cultivars Svevo and Ciccio. Mapping data localized *GS2* and *GSe* genes on chromosomes 2A, 2B, (*GS2*) and 4A, 4B (*GSe*) where four significant QTLs for GPC where found by Blanco *et al*. (2012).

The high sequence homology was found for plant cytosolic and plastidic *GS* as also reported by Bernard *et al*. (2008) suggesting that they are derived from a common ancestor, and providing molecular evidence supporting the mechanism of chloroplast evolution (Weeden, 1981). This model proposed that genes for plastid isozymes evolved by duplication of nuclear genes and subsequent specialization of each locus.

The genomic sequences of the two homoeologous A, and B *NADH-GOGAT* genes were obtained in the same durum wheat cultivars by Nigro *et al*. (2013). Analysis of the gene sequences indicates that all wheat *NADH-GOGAT* genes are composed of 22 exons and 21. The two hexaploid wheat homoeologous genes show the same exon/intron number and size except intron 13 which shows differences in both length and sequence for all of three homoeologues. A comparative analysis of sequences has been conducted among di- and mono-cotyledous plants and shows both regions of high conservation and of divergence.

qRT-PCR performed with the two durum wheat cvs Svevo and Ciccio (characterized by an high and low protein content, respectively) was conducted for the three genes (*GS2*, *GSe* and *GOGAT*).

Total RNA extracted from plants grown in field conditions and reverse-transcribed for qRT-PCR analyses. To test if the homoeologous genes show differential expression patterns, qRT-PCR were performed using specific primers designed to preferentially amplify the A and B sequences for the six genes in leaves (at seedling stage) at three different phenotypic stages (first leaf, flowering and grain filling).

Different expression levels of the two *NADH-GOGAT-3A* and *NADH-GOGAT-3B* genes was observed, transcript levels in the first leaf and grain filling stages showed similar expression levels, while a significantly higher value of transcripts was observed during flowering ($P<0.01$) (Nigro *et al*., 2013). A similar trend was observed for both the homoeologous genes and in the two cultivars (Fig.1).

The physical chromosome position of the *NADH-GOGAT-3B* gene co-localize with Meta QTLs for high protein content reported by Quraishi *et al*. (2011). They showed, a NUE QTL conserved at the same orthologous loci as the *GOGAT* gene on wheat chromosome 3B, rice chromosome 1, sorghum chromosome 3 and maize chromosomes 3 and 8, despite 50–70 million years of separate evolution associated with considerable sequence shuffling. For these reasons *NADH-GOGAT* is one of the potential candidate genes involved in the control of the complex character trait GPC.

The transcription level of the *GSe* and GS2 was also investigated. A significant different expression was observed for both genes between the two cvs. Higher values of expression were observed for *GSe-A4* during the flowering time, while the higher value of *GSe-B4* expression was observed during the maturation, indicating that the homoeologous alleles play non overlapping roles in the different phenological phases and that alleles encoded by “Svevo” are more expressed than the “Ciccio” ones, probably due to differences in the promoter region or to a different gene regulation between the two cvs Ciccio and Svevo (data reported in Gadaleta *et al*., 2014).

Figure 1. Comparison of the expression level of NADH-GOGAT-3A and NADH-GOGAT-3B genes in three different phenological phases (first leaf, flowering, grain filling) of cv Ciccio and Svevo.

A different trend was observed for *GS2-B2* whose transcript level increased during the three different phenological phases with a major value during maturation. ANOVA showed highly significant differences ($P<0.001$) between the two cultivars also for GS2-B2 gene during flowering and maturation (Fig.2). In conclusion we can say that in the present work candidate gene approach was efficiently applied for the study of grain protein content ion wheat.

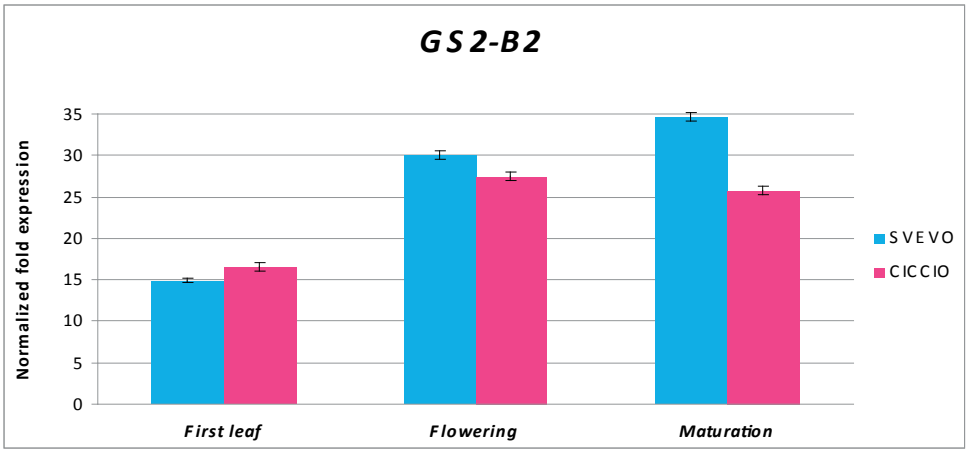

Figure 2. qRT-PCR conducted for GS2-B2 gene with specific probes in three different phenological phases (first leaf, flowering, grain filling) of cv Ciccio and Svevo.

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References

Autran J.C., Abecassis J., Feillet P., 1996. Statistical evaluation of different technological and biochemical tests for quality assessment in durum wheats. *Cereal Chem*., 63, pp. 390-394.

Baron A., Tobin A., Wallsgrove R.M., 1994. The kinetics of azaserine and phosphinothricin inhibition of glutamate synthase cycle enzymes from barley leaves. *Plant Physiol. Biochem*., 32, pp. 555–560.

Bernard S., Habash D.Z., 2009. The importance of cytosolic glutamine synthetase in nitrogen assimilation and recycling. *New Phytologist.,* 182, pp. 608-620.

Blanco A., Pasqualone A., Troccoli A., Di Fonzo N., Simeone R., 2002. Detection of grain protein content QTLs across environments in tetraploid wheats. *Plant Molecular Biology,* 48, pp. 615–623.

Blanco A., Simeone R., Gadaleta A., 2006. Detection of QTL for grain protein content in durum wheat. *Theor. Appl. Genet*., 112, pp. 1195–1204.

Blanco A., Mangin, 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, pp. 79-92.

Börner A., Schumann E., Furste A., Coster H., Leithold B., Röder M.S., Weber W.E., 2002. Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). *Theor. Appl. Genet*., 105, pp. 921–936.

Brugie`re N., Dubois F., Limami A., Lelandais M., Roux Y., Sangwan R., Hirel B., 1999. Glutamine synthetase in the phloem plays a major role in controlling proline production. *Plant Cell,* 11, pp. 1995-2011.

Campbell K.G., Finney P.L., Bergman C.J., Gualberto D.G., Anderson J.A., Giroux M.J., Siritunga D., Zhu J., Gendre F., Roue C., Verel A., Sorrells M.E., 2001. Quantitative trait loci associated with milling and baking quality in a soft×hard wheat cross. *Crop Sci.,* 41, pp. 1275–1285.

Canovas F., Avila C., Canton F.R., Canas R., de la Torre F., 2007. Ammonium assimilation and amino acid metabolism in conifers. *J. Experimental Botany,* 58, pp. 2307-2318.

Chen F.L., Cullimore J.V., 1989. Location of two isoenzymes of NADH-dependent glutamate synthase in root nodules of Phaseolus vulgaris. *Planta*, 179, pp. 441–447.

Dholakia B.B., Ammiraju J.S.S., Sandra D.K., Singh H., Katti M.V., Lagu M.D., Tamhankar S.A., Rao V.S., Gupta V.S., Dhaliwal H.S., Ranjekar P.K., 2001. Molecular marker analysis of protein content using PCR–based marker in wheat. *Biochem. Genet*., 39, pp. 325–338.

Finnemann J., Schjoerring J.K., 2000. Post-translational regulation of cytosolic glutamine synthetase by reversible phosphorylation and 14-3-3 protein interaction. *Plant. J.,* 24, pp. 171-181.

Gadaleta A., Nigro D., Giancaspro A., Blanco A., 2011. The glutamine synthetase *(GS2)* genes in relation to grain protein content of durum wheat. *Functional and Integrative Genomics,* 11, pp. 665-670.

Gadaleta A., Giancaspro A., Nigro D., Giove S.L., Zacheo S.A., Incerti O., Simeone R., Piarulli L., Colasuonno P., Blanco A., 2014. A new genetic and deletion map of wheat chromosome 5A to detect candidate genes for quantitative traits. *Molecular Breeding,* Doi: 10.1007/s11032-014-0185-1.

Galais A., Hirel B., 2004. An approach to the genetics of nitrogen use efficiency in maize. *J. Exp. Bot.,* 55, pp. 295-306.

Gebhardt C.G., Li L., Pajerowska-Mukthar K., Achenbach U., Sattarzadeh A., Bormann C., Ilarionova E., Ballvora A., 2007. Candidate gene approach to identify genes underlying quantitative traits and develop diagnostic markers in potato. *Crop Sci.,* 4, pp. 106-111.

Gonzalez-Hernandez J.L., Elias E.M., Kianian S.F., 2004. Mapping genes for grain protein concentration and grain yield on chromosome 5B of *Triticum turgidum* (L.) var. *dicoccoides*. *Euphytica,* 139, pp. 217–225.

Groos C., Robert N., Bervas E., Charmet G., 2003. Genetic analysis of grain protein content, grain yield and thousand–kernel weight in bread wheat. *Theor. Appl. Genet*., 106, pp. 1032–1040.

Habash D.Z., Massiah A.J., Rong H.L., Wallsgrove R.M., Leigh R.A., 2001. The role of cytosolic glutamine synthetase in wheat. *Ann. Appl. Biol*., 138, pp. 83-89.

Hayakawa T., Nakamura T., Hattori F., Mae T., Ojima K., Yamaya T., 1994. Cellular localization of NADH-dependent glutamate synthase protein in vascular bundles of unexpanded leaf blades and young grain of rice plants. *Planta*, 193, pp. 455-460.

Hirel B., Lea P.J., 2001. Ammonia assimilation. In: Lea PJ, Morot-Gaudry J-F (ed) *Plant nitrogen*. Springer-Verlag, Berlin, pp. 79-99.

Hirel B., Le Gouis J., Ney B., Gallais A., 2007. The challenge of improving nitrogen use efficiency in crop plants : towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J. Experimental Botany,* 58, pp. 2369-2387.

Huang X.Q., Cloutier S., Lycar L., Radovanovic N., Humphreys D.G., 2006. Molecular detection of QTLs for agronomic and quality traits in a doubled haploid population derived from two Canadian wheats (*Triticum aestivum* L.). *Theor. Appl. Genet*., 113, pp. 753–766.

Ireland R.J., Lea P.J., 1999. The enzymes of glutamine, glutamate, asparagines and aspartate metabolisms. In:. *Plant amino acids: Biochemistry and biotechnology.* Singh B.K. (ed), New York: Marcel Dekker, pp. 49-109.

Joppa L.R., Cantrell R.G., 1990. Chromosomal location of genes for grain protein content of wild tetraploid wheat. *Crop Sci*., 30, pp. 1059—1064.

Joppa L.R., Du C., Hart G.E., Hareland G.A., 1997. Mapping genes for grain protein in tetraploid wheat (*Triticum turgidum* L) using a population of recombinant inbred chromosome lines. *Crop Sci*., 37, pp. 1586–1589.

Kamachi K., Yamaya T., Mae T., Ojima K., 1991. A role for glutamine synthetase in the remobilization of leaf nitrogen during natural senescence in rice leaves. *Plant Physiol*., 96, pp. 411-417.

Khan I.A., Procunier J.D., Humphreys D.G., Tranquilli G., Schlatter A.R., Marcucci–Poltri S., Frohberg R., Dubcovsky J., 2000. Development of PCR–based markers for a high grain protein content gene from *Triticum turgidum* ssp. *dicoccoides* transferred to bread wheat. *Crop Sci*., 40, pp. 518–524.

Konzak C.F., 1977. Genetic control of the content, aminoacid composition and processing properties of proteins in wheat. *Adv. Gen*., 19, pp. 407–582.

Kramer T.H., 1979. Environmental and genetic variation for protein content in winter wheat (*Triticum aestivum*). *Euphytica,* 28, pp. 209-218.

Krapp A., Saliba-Colombani V., Daniel-Vedele F., 2005. Analysis of C and N metabolisms and of C/N interactions using quantitative genetics. *Photosynth. Res*., 83, pp. 251–263.

Levy A.A., Feldman M., 1989. Location of genes for high grain protein percentage and other quantitative traits in wild wheat, *T. turgidum* var. *dicoccoides*. *Euphytica,* 41, pp. 113–122.

Mann G., Diffey S., Cullis B., Azanza F., Martin D., Kelly A., McIntyre L., Schmidt A., Ma W., Nath Z., Kutty I., Leyne P.E., Rampling L., Quail K.J., Morell M.K., 2009. Genetic control of wheat quality: interactions between chromosomal regions determining protein content and composition, dough rheology, and sponge and dough baking properties. *Theor. Appl. Genet*., 118, pp. 1519–1537.

McNally S., Hirel B., Gadal P., Mann A.F., Stewart G.R., 1983. Glutamine synthetases of higher plants-evidence for specific isoform content related to their possible physiological role and their compartmentation within the leaf. *Plant Physiology,* 72, pp. 22-25.

Nelson J.C., Andreescu C., Breseghello F., Finney P.L., Gualberto D.G., Bergman C.J., Pena R.J., Perretant M.R., Leroy P., Qualset C.O., Sorrells M.E., 2006. Quantitative trait locus analysis of wheat quality traits. *Euphytica,* 149, pp. 145–159.

Nigro D., Gu Y.Q., Huo N., Marcotuli I., Blanco A., Gadaleta A. , Anderson O.D., 2013. Structural Analysis of the Wheat Genes Encoding NADH-Dependent Glutamine-2-oxoglutarate Amidotransferases and Correlation with Grain Protein Content. *PLoS ONE*, 8 (9): e73751.

Obara M., Sato T., Sasaki S., Kashiba K., Nagano A., Nakamura I., Ebitani T., Yano M., Yamaya T., 2004. Identification and characterization of a QTL on chromosome 2 for cytosolic glutamine synthetase content and panicle number in rice. *Theor. Appl. Genet*., 110, pp. 1-11.

Olmos S., Diestelfeld A., Chicaiza O., Schatter A.R., Fahima T., Echenique V., Dubcovsky J., 2003. Precise mapping of a locus affecting grain protein content in durum wheat. *Theor. Appl. Genet*., 107, pp. 1243–1251

Pajerowska K.M., Parker J.E., Gebhardt C., 2005. Potato homologs of Arabidopsis thaliana genes functional in defence signalling- identification, genetic mapping, and molecular cloning. *Molecular Plant-Microbe Interactions,* 18, pp. 1107-1119.

Pereira A.B., Pedras J.F., Villa Nova N.A., Cury D.M., 1995. Water consumption and crop coefficient of potato (Solanum tuberosum L.) during the winter season in municipality of Botucatu-SP. *Rev. Bras. Agrometeorol*., 3, pp. 59-62.

Perretant, M.R., Cadalen, T., Charmet, G., Sourdille, P., Nicolas, P., Boeuf, C., Tixier, M.H., Branlard, G., Bernard, S., Bernard, M. (2000). QTL analysis of bread–making quality in wheat using a doubled haploid population. *Theor. Appl. Genet*. 100, pp.1167–1175

Prasad M., Varshney R.K., Kumar A., Balyan H.S., Sharma P.C., Edwards K.J., Singh H., Dhaliwal H.S., Roy J.K., Gupta P.K., 1999. A microsatellite marker associated with a QTL for grain protein content on chromosome 2DL of bread wheat. *Theor. Appl. Genet*., 99, pp. 341–345.

Prasad M., Kumar N., Kulwal P.L., Roder M.S., Balyan H.S., Dhaliwa, H.S., Roy J.K., Gupta P.K., 2003. QTL analysis for grain protein content using SSR markers and validation studies using NILs in bread wheat. *Theor. Appl. Genet*., 106, pp. 659–667.

Quraishi U.M., Abrouk M., Murat F., Pont C., Foucrier S., Desmaizieres G., Confolent C., Rivière N., Charmet G., Paux E., Murigneux A., Guerreiro L., Lafarge S., Le Gouis J., Feuillet C., Salse J., 2011. Cross-genome map based dissection of a nitrogen use efficiency ortho-meta QTL in bread wheat unravels concerted cereal genome evolution. *The Plant Journal,* 65, pp. 745-756.

Raman R., Allen H., Raman H., Diffey S., Martin P., McKelvie K., 2009. Localisation of quantitative trait loci for quality attributes in a doubled haploid population of wheat (*Triticum aestivum L*.). *Genome,* 52, pp. 701-715.

Sakurai N., Hayakawa T., Nakamura T., Yamaya T., 1996. Changes in the cellular localization of cytosolic glutamine synthetase protein in vascular bundles of rice leaves at various stages of development. *Planta,* 200, pp. 306-311.

Snape J.W., Hyne V., Aitken K., 1995. Targeting genes in wheat using marker–mediated approaches. In: *Proceeding 8th International Wheat Genetics Symposium.* Li Z.S., Xin Z.Y. (eds), Beijing, 20–25 July 1993, China Agric Scientech Press, Be jing, China, pp. 749–759.

Stein I.S., Sears R.G., Hoseney R.C., Cox T.S., Gill B.S., 1992. Chromosomal location of genes influencing grain protein concentration and mixogram properties in Plainsman V winter wheat. *Crop Sci*., 32, pp. 573–580.

Sourdille P., Perretant M.R., Charmet G., Leroy P., Gautier M.F., Joudrier P., Nelson J.C., Sorrells M. E., Bernard M., 1996. Linkage between RFLP markers and gene affecting kernel hardness in wheat. *Theor. Appl. Genet*., 93, pp. 580–586.

Sun Y., Wang J., Crouch J.H., Xu Y., 2010. Efficiency of selective genotyping for genetic analysis of complex tarits and potential applications in crop improvement. *Mol. Breed.,* 26, pp. 493–511.

Suprayogi Y., Pozniak C.J., Clarke F.R., Clarke J.M., Knox R.E., Singh A.K., 2009. Identification and validation of quantitative trait loci for grain protein concentration in adapted Canadian durum wheat populations. *Theor. Appl. Genet*., 119(3) , pp. 437–448.

Tabuchi M., Sugiyama K., Ishiyama K., Inoue E., Sato T., Takahashi H., Yamaya T., 2005. Severe reduction in growth rate and grain filling of rice mutants lacking *OsGS1;1*, a cytosolic glutamine synthetase. *Plant Journal,* 42, pp. 641-651.

Tobin A.K., Ridley S.M., Stewart G.R., 1985. Changes in the activities of chloroplast and cytosolic isoenzymes of glutamine synthetase during normal leaf growth and plastid development in wheat. *Planta,* 163, pp. 544-548.

Turner A.S., Bradburne R.P., Fish L., Snape J.W., 2004. New quantitative trait loci influencing grain texture and protein content in bread wheat. *J. Cereal Sci*., 40, pp. 51–60

Weeden N.F., 1981. Genetic and biochemical implications of the endosymbiotic origin of the chloroplast. *Journal of Molecular Evolution,* 17, pp. 133-139.

Yamaya T., Obara M., Nakajima H., Sasaki S., Hayakawa T., Sato T., 2002. Genetic manipulation and quantitative-trait loci mapping for nitrogen recycling in rice. *J. Exp. Bot*., 53, pp. 917-925.

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. *Theor. Appl. Genet*., 117, pp. 1361-1377.

Zanetti S., Winzeler M., Feulillet C., Keller B., Messmer M., 2001. Genetic analysis of bread–making quality in wheat and spelt. *Plant Breed*., 120, pp. 13–19.