ASSOCIATION MAPPING IN BRAD WHEAT: APPLICATION TO QUALITY TRAITS

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Triticum aestivum, protein composition, micro-nutrients, dietary fibre

A brief introduction is given about the basics of association genetics and the key factors that influence its power and resolution. Definition of linkage disequilibrium and the parameters that affect its range are presented. Sources of spurious associations such as population structures of genetic relatedness are discussed, as well as common tools used for correction. Then three examples of application to bread wheat quality traits illustrate three different uses of association genetics.

1) A candidate gene approach with an application to storage protein composition, which enabled to decide among two candidates located within a 1.3 cM region.

2) A chromosome scan approach, which enabled us to confirm QTLs for dietary fibre.

3) A genomicscan approach using DArT markers to identify chromosomal regions involved in micronutrient contents.

Based on real datasets, some simulation results are also used to discuss type I and type II error risks in genomewide analyses.
ASSOCIATION MAPPING OF FROST TOLERANCE QTL IN BARLEY


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association mapping, Hordeum vulgare, frost tolerance

A better understanding of the genetics of frost tolerance could have a significant impact on world food supply, since low-temperature-related stresses limit the productivity of many plants of agronomic and horticultural value. Barley (Hordeum vulgare) is an excellent model system to unravel the genetic bases of frost tolerance, because of the large variation for this trait within the primary gene pool and an ever-expanding set of tools for genome analysis. Within the ERA-PG funded project ExBarDiv (Genomics-assisted analysis and exploitation of barley diversity) three different populations - namely cultivar, landrace and wild (Hordeum spontaneum) germplasm collections - have been assembled in order to test the efficiency of an incremental association mapping approach for identifying new useful gene alleles. As a first step within this approach, here we report the evaluation of frost tolerance in 285 barley spring cultivars. For each accession, 8 first-leaf stage plants have been cold acclimated for 4 weeks (3°C, 8 h light and 2°C, 16 h dark), then exposed to two different freezing conditions (-12°C and -10°C) for 10 h. To evaluate the effect of freezing on the functionality of the Photosystem II (PSII) reaction centers, the maximum quantum yield of the PSII photochemistry has been measured by the ratio of variable (Fv) to maximal (Fm) fluorescence in a dark-adapted state (Fv/Fm), using a Pulse Amplitude-Modulated fluorometer, after a 24 h recovery time. The same germplasm collection has been genotyped with 1536 gene-based SNPs using the Illumina™ OPA (oligo probe assay) high throughput marker technology. Molecular marker information will be used to determine the underlying population structure and perform association analyses between the phenotype and genotype data sets. Broad genomic regions containing potentially useful gene alleles for barley frost tolerance will be presented.
DISCOVERY OF A NON-SYNONYMOUS MUTATION OF 1-DEOXY-D-XYLULOSE-5-PHOSPHATE SYNTHASE 1 GENE ASSOCIATED WITH MUSCAT FLAVORED GRAPE VARIETIES

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candidate gene, dxs-1, monoterpenes, structured association, Vitis vinifera

Flavor has been described as a domestication-related trait in grape, together with hermaphroditism, large seeds/fruit and berry color. The typical aroma of Muscat grapes is strongly-coupled with the presence of monoterpenols. Based on the accumulation of linalool, geraniol, and nerol in two segregating populations we identified a stable major QTL on linkage group 5 with a LOD maximum at the 1-deoxy-D-xylulose-5-phosphate synthase 1 (dxs-1) locus. DXS is an important regulatory enzyme of the mevalonate-independent pathway involved in terpenoid biosynthesis, thus dxs-1 is a strong candidate gene for muscat flavor determination. We investigated the nucleotide variability of dxs-1 by exploiting the natural genetic variation of a grape collection. A set of 148 grapevines which displayed the maximum of diversity for the trait and retained at least 80% of the microsatellite diversity found within Vitis vinifera was considered for the study. The predicted dxs-1 gene was re-sequenced in all the accessions under study and the full-ORF cDNAs of the two Muscat Blanc dxs-1 alleles were cloned and sequenced in order to precisely define exons and introns. Ninty-five haplotypes were reconstructed considering 101 polymorphic sites and a network analysis revealed a major star-shaped cluster of dxs-1 haplotypes present only in Muscat genotypes. Using statistical models that correct for population structure, a heterozygous non-synonymous mutation in a highly conserved region of the gene was found to be strongly associated with Muscat genotypes and to characterize the Muscat haplogroup. Moreover, muscat-like aromatic mutants showed unique heterozygous non-synonymous mutations close to the mutated site of Muscat genotypes. These results support a major role of dxs-1 in muscat flavor determination, which is currently under validation through expression analysis and functional assays.
LEVERAGING LINKAGE AND ASSOCIATION MAPPING TO IDENTIFY AGRONOMICALLY VALUABLE ALLELES IN DURUM WHEAT


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Association mapping, Triticum durum Desf., disease resistance, molecular haplotypes

At DiSTA and PSB, in collaboration with European and West-Asian and North African (WANA) research Institutions, CIMMYT and ICARDA, a program for mapping useful genetic variation in durum wheat through a joint linkage and association mapping approach has been undertaken since 2003. Up to now, three recombinant inbred line mapping population obtained from the crosses Kofa x Svevo, Meridiano x Claudio and Colosseo x Lloyd (each including ca. 180 RILs) and a germplasm collection of 210 cultivated durum wheat accessions have been characterized with molecular markers in a genome-wide approach and phenotyped under a range of environments for adaptive and agronomically relevant traits, kernel quality traits and response to the major wheat diseases such as leaf rust, powdery mildew and soil-borne cereal mosaic virus.

The durum wheat germplasm collection includes for the most semi-dwarf elite materials released from early ’70s up to late ’90s and largely covers the genetic variation present in the main durum wheat breeding programs.

Association mapping with this germplasm collection has been used to gather more information on the effects of the QTLs identified in the mapping populations as above and offered the opportunity to investigate the allele effects across a broad range of genetic backgrounds and the presence of valuable alleles in the germplasm. Population structure analysis showed the presence of five main subpopulations, corresponding to important breeding lineages and durum ideotypes. A linkage disequilibrium (LD) decay rate survey based on 180 mapped SSR loci showed high LD levels ($r^2 > 0.20$ and $D' > 0.60$) within a 5-10 cM (maximum) inter-marker distance.

Examples of the use of the germplasm collection for QTL dissection studies include: a major gene for leaf rust resistance on chr. 7BL (Colosseo x Lloyd), two major QTLs for grain yield and related traits on chr.s 2BL and 3BS (Kofa x Svevo) and one major QTL for soil borne cereal mosaic virus on chr. 2BS (Meridiano x Claudio). Additionally a comprehensive analysis of the genetic basis of kernel yellow pigment content in the three populations and in the germplasm collection allowed us to evaluate allelic variation and germplasm diversity at some of the major QTLs controlling the quality trait.

The DISTA durum association mapping panel is being directly used also in a genome-wide association mapping scanning using high-throughput genotyping approaches such as DArT and SNP markers. A subset of founder accessions from the germplasm collection have been used by Triticarte™ to develop the specific 2.0 Durum wheat PsT I (Taq I) DArT array including more than 2,000 polymorphic markers. Durum specific SNP marker assays suitable for genotyping in the Illumina VeraCode technology platform are being developed by KeyGene (Wageningen, Netherland) within the EU-funded BIOEXPLOIT project.
GENETICS OF YELLOW PIGMENT CONTENT IN DURUM WHEAT AS ASSESSED VIA LINKAGE AND ASSOCIATION MAPPING


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Grain yellow pigment content (GYP, mostly based on luteins) is an important quality trait in durum wheat, where a bright yellow colour is a standard quality pre-requisite for semolina and pasta products. Although GYP is a quantitative trait with a relatively high heritability, a considerable portion of the cultivated germplasm still has GYP lower than the market requirements. On the same line, high GYP donors have been identified (even in the cultivated germplasm) and are being actively exploited in breeding programs. Thus, it would be advisable to identify the major QTLs controlling the traits and tag them with molecular markers amenable to marker-assisted selection.

We studied GYP in the cultivated durum wheat in a comprehensive genetic study including three RIL populations (Kofa x Svevo, KS, with 249 RILs; Colosseo x Lloyd, CL, with 176 RILs, and Meridiano x Claudio, MC, with 181 RILs) and a germplasm collection (Durum Panel including 189 cultivated durum accessions bred for Mediterranean areas and a panel of North American durums) suitable for association mapping. These materials were grown in Italy and in other Mediterranean countries over several years and locations and whole grain yellow index was determined as b* (Minolta b-value). Linkage maps for the three RIL populations were obtained with SSR and DArT markers from the Triticarte Durum Array v 2.0. The average intermarker distance was of 5 cM. The Durum Panel was genotyped with 180 SSRs of known map position (as from the RIL linkage maps) and with 900 DArT markers.

Major QTLs from one or two populations (average $R^2 > 5\%$ using mean data) were found in the following chr. regions: chr. 1AS (CL population and Durum Panel), chr. 4AL (KS population), chr. 4BL (KS and CL populations and Durum Panel), chr. 5BL (KS and CL populations), chr. 6Ac (KS, CL populations and Durum Panel), chr. 7AL (Durum Panel), chr. 7BS (KS, CL, MC populations and Durum Panel) and chr. 7BL (Durum Panel). Several minor QTLs, showing specificity for one single population, were also identified.

The detailed results of the QTL and association analysis will be presented and discussed.
CANDIDATE GENE-BASED ASSOCIATION MAPPING FOR MAIN AGRONOMIC TRAITS IN DURUM WHEAT IN DIFFERENT WATER REGIMES


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Association mapping, stress resistance, durum wheat

Association or linkage disequilibrium (LD) mapping is a strategy to identify associations between the alleles or haplotypes present in natural populations or collections of germplasm and the trait of interest. Marker-trait associations can be found either saturating the genome with markers (whole genome scan) or looking at variation in specific genes (candidate gene association mapping). A candidate gene association study was carried out to identify genes that are involved in the genetic control of traits related to grain yield in different water regimes in durum wheat. Candidate genes were selected on the basis of sequence similarity and expression data available in literature. Sequences coding for transcription factors and other proteins involved in post-transcriptional regulation were selected. These genes were preliminarily sequenced on 12 inbred lines, that greatly differ in terms of genetic diversity and stress resistance in order to verify the presence of polymorphic sites in the candidate gene sequence. This preliminary analysis revealed the presence of polymorphic sites in 26 out of 72 genes sequenced resulting in a SNP frequency of 0.17%. Moreover, no significant decay of LD was observed in the sequenced regions. The polymorphic genes were then sequenced on the rest of the inbred lines that were characterized phenotypically during the project (84 inbred lines in total), and an association analysis was carried out between 22 polymorphisms able to distinguish the haplotypic variations in the germplasm and six phenotypic traits (grain yield, heading date, plant height, thousand-kernel weight, test weight, and protein content) determined in 14 environments characterized by different soil and climatic conditions. The analysis was performed by considering the population structure. Results of the association tests and of the further characterization of the genes associated with the phenotype are presented.
HIGH-THROUGHPUT GENOTYPING AND HIGH-RESOLUTION PHENOTYPING FOR A COMPREHENSIVE QTL MAPPING RELATED TO APPLE FRUIT CRISPNESS


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fruit quality, high resolution phenotyping, high-throughput markers genotyping, QTL mapping

In the definition of fruit quality in apple, crispness is certainly the major characteristic. Crispness, which is associated to the cell wall disruption mechanism and turgor pressure, is perceived as emitted sound during compression.

Besides its sensorial perception, a crispy apple is generally more appreciated because its higher flavour and aroma release.

In this context we performed a pilot study aimed to discovery the QTLs putatively involved in the control of the “crispy” phenotype.

To perform our investigation we genotyped two mapping populations, Fuji x Delearly and Fuji x Pink Lady, with two type of molecular markers. First, a series of SSR (CH and Hi series) were extended in a multiplex system with an ABI 3730 DNA analyzer, to build up the maps scaffold, necessary for linkage groups comparison with other reference maps.

The second category was represented by a set of SNP markers (ad hoc identified between the two haplomes of the heterozygous Golden Delicious genome), genotyped in high throughput using SNPllex™ (Applied Biosystem) and Golden Gate genotyping assay (Illumina).

High resolution phenotyping, addressed to dissect most of the fruit flesh complexity, was carried out analyzing a series of acoustic and mechanical parameters via a TA.XT Texture Analyzer instrument (Stable Micro Systems) equipped with an acoustic detector.

The preliminary QTL mapping study identified significative genomic regions on these two populations possibly involved in the control of fruit crispness and firmness. These regions will be further explored in order to identify the gene set included in the QTL interval, with the final aim to investigate the allele mining of these future candidate genes in a wider apple collection.
LEAF RUST RESISTANCE GENES/QTLs IN DURUM WHEAT INVESTIGATED VIA ASSOCIATION MAPPING APPROACHES

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Association mapping, durum wheat, leaf rust resistance, molecular haplotypes

Leaf rust (Puccinia triticina) is a main fungal disease for durum wheat worldwide. Association mapping based on germplasm collections has been recently introduced in crops to validate the effect of genes/QTLs previously discovered with traditional mapping and/or to discover novel valuable allelic variation. In this study, a panel of 200 elite durum wheat accessions (Durum Panel) representative of the genetic variation present in the Mediterranean Basin and in CIMMYT and ICARDA breeding programs were tested for leaf rust response at adult plant and at seedling stage. Field experiments were carried out in artificially inoculated field trials in Italy (Argelato, Bologna) in 2006 and 2007 and in Mexico (Obregon and El Batan) in 2006, 2007 and 2008; infection type (IT) at the seedling stage was evaluated by means of artificial inoculation of 18 single spore P. triticina isolates in greenhouse experiment carried out in Italy, Minnesota, Poland and Israel. The Durum Panel was also profiled with ca. 200 SSRs and ca. 900 DArT markers of known genetic map position (linkage groups and genetic distances determined from a mapping effort using durum wheat recombinant inbred line populations). Moreover, the genetic region on chr. 7BL (deletion bin 0.78-1.00) known to harbour the major gene for leaf rust resistance present in Creso (QLr.ubo-7B.2, Maccaferri et al., 2008, TAG 117: 225-40) and the resistance gene Lr14a identified in cv. Llareta (Herrera-Foessel et al., 2008, Plant Dis 92, 469-473) has been investigated in detail through haplotyping with 16 SSR markers.

Association mapping revealed that the locus present in the 7BL region is the most important source of resistance exploited by breeders to obtain durum materials for the Mediterranean areas. Based on the profiles of four SSRs mapped in the 7BL region and showing the highest association with leaf rust resistance, the haplotypes of Creso and Llareta are most probably identical by descent; further, the Creso/Llareta haplotype is present in a number of resistant accessions from CIMMYT and ICARDA breeding programs.

In a genome-wide association mapping approach, a separate analysis of the accessions not carrying the Creso/Llareta haplotype at the 7BL region and the use of four isolates which overcome the Creso-resistance allowed us to map other regions most probably harbouring useful alleles for leaf rust resistance response.
QTL MAPPING FOR PEACH (PRUNUS PERSICA L. BATSCH) RESISTANCE TO POWDERY MILDEW AND BROWN ROT

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QTL analysis, Powdery mildew, Brown rot, disease resistance, Prunus persica

Powdery Mildew (PM, caused by the biotrophic Sphaerotheca pannosa) and Brown Rot (BR, caused by the necrotrophic Monilinia spp.) are important diseases in Northern Italy peach orchards, giving place to major losses at pre- and post-harvest level. Previous works showed the possibility to discriminate between tolerant and susceptible peach genotypes for both diseases, suggesting a quantitative nature of their respective resistances. In order to discover genetic markers for these traits, two F1 populations were selected to be used in SSR-based QTL mapping of both resistances: one from the cross of the cultivars Bolero x OroA, segregating for PM resistance (BxO, 129 individuals); and other from the cross Contender x Elegant Lady, segregating for BR resistance (CxEL, 110 individuals). PM resistance of BxO population was evaluated by infection of peach leaves over four years, and showed high data reproducibility and normal distribution, in which the parents Bolero and OroA were at its susceptible and tolerant extreme, respectively. On the other hand, BR resistance of CxEL population was analyzed using conidial spraying inoculation of harvested fruits for three years, showing a low data reproducibility, a high proportion of susceptible genotypes, and a very low proportion of highly tolerant individuals, some of them being more resistant than Contender, the tolerant parent. In order to select heterozygous markers for the construction of the linkage maps, and an initial pool of 344 SSRs were analyzed in the 4 parents. From the 317 SSRs that amplified successfully 81, 47, 87 and 85 resulted to be heterozygous in Bolero, OroA, Contender and Elegant Lady, respectively. The maps were generated with 26 SSRs for Bolero, 16 for OroA, 43 for Contender and 41 for Elegant Lady, covering from 25.03% (OroA) to 67.5% (CxEL) of the total genetic distance of the TxE reference map. The QTL analyses were performed using Interval Mapping (IM), and its the genome-wide LOD threshold was determined using permutation tests. For the PM resistance, three QTLs were found on the LG1, LG5 and LG7 of the OroA genome (the tolerant parent), explaining from 6 to 8% of the phenotypic variation. One of these QTLs, located in the chromosome 7 was consistent in two years of analysis, and confirmed a previously reported QTL (Foulongne et al., 2003). For the BR resistance however, we did not obtain regions with a significant LOD score, possibly because of its strong dependence on the climatic conditions of the year of analysis and the presence of latent infections on the analyzed fruits. This suggests the necessity of the designing of a new strategy to measure the resistance to BR.
ASSOCIATION OF POLYMORPHIC RAPD AND ISSR MOLECULAR MARKERS TO PHENOTYPIC DIFFERENCES FOR PLANT PHENOLOGY, SEED SIZE AND BIOTIC STRESS RESISTANCE TRAITS IN HAZELNUT (
\textit{Corylus avellana} L.)

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Association mapping, MAS, Bulk segregant analysis

Marker assisted selection (MAS) speed-up the choice of novel allelic combination in genotypes from segregating population, especially during fruit- crop breeding. However, the efficacy of MAS depends on the availability of basic information related to the mapped molecular markers and the molecular variants associated to the target trait phenotype chosen as selection criteria. In the case of hazelnut such information are not on hand yet. Therefore, we undertook an association mapping study in the near full-sib (FS) progeny obtained from crossing “Tonda Gentile Romana” (TGR) and “Nocchione” (NOC), the two most important hazelnut landraces grown in the Latium region of Italy.

The first step was the identification of two divergent groups of FS plants displaying extreme phenotypes for each of the traits related to plant phenology (time of beginning of bud sprouting and time of beginning of anthesis), seed size (seed weight) and biotic stress resistance (big bud mite resistance). These groups contained plants that gave repeatable and significant divergent three-year mean values for the mentioned traits. DNA was purified from each plant in each divergent group and bulked in order to have a DNA-bulk from the divergent group of plants with high (DB-H) and low (DB-L) phenotypic values, respectively.

The second step has been the identification of RAPD and ISSR molecular markers that showed polymorphism between the parental landraces. RAPD and ISSR primers were chosen on the basis of the high polymorphism they detected in forest tree genera phylogenetically closer to hazelnut, such as \textit{Castanea} and \textit{Notophagus} We found interparental polymorphism for 43% of the tested RAPD primers and for 23% of the ISSR primers tested.

The third step involved the analysis of association between specific RAPD or ISSR polymorphic bands and one of the two bulks of DNA. About 1/3 of the polymorphic RAPD markers showed differences between the DNA bulks. Nevertheless, in various cases the same RAPD primer exhibited interbulk polymorphism for more than one trait, which prevented its usefulness in defining unambiguous association to major quantitative trait loci for single traits. These results suggested to include multilocus molecular markers (i.e. AFLP) to accelerate discovery of markers associated to divergent phenotypes for single traits.
PHYTOENE SYNTHASE 2 LOCI (PSY2) IN DURUM WHEAT - MOLECULAR CHARACTERIZATION AND DEVELOPMENT OF FUNCTIONAL MARKERS FOR SEMOLINA COLOUR

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phytoene synthase 2, durum wheat, yellow pigment

Yellow pigment content in durum wheat (Triticum turgidum L. ssp. durum) is an essential feature for both pasta yellow color and human health because of antioxidant properties of carotenoids involved in the yellow pigmentation.

Carotenoids are the most important components of semolina yellow pigments and are synthesized through a complex pathway, involving more than 10 enzymatic steps. Phytoene synthase (Psy), catalyzing the condensation of two geranylgeranyl pyrophosphate molecules into phytoene, is generally considered the rate-limiting enzyme in carotenoids biosynthesis. In the grass family, triplicated genes were identified and designated as Psy1, Psy2, and Psy3 respectively. Phytoene synthase 1 showed effective association with the yellow pigment (YP) content in wheat grain. Characterization of Psy genes and development of functional markers are important for marker-assisted selection in wheat breeding.

One of the goals of this study is to develop functional markers based on the sequence of Psy2 gene sand to map them in different segregant populations and a set of Chinese Spring nullitetrasomic, ditelosomic and deletion lines. To date no full-length DNA sequence of Psy2 has been cloned in either common or durum wheat. With the aim of clarifying the structure of Psy2 gene in the coding sequence region and identifying allelic variants at this locus, the CDS region was sequenced by cloning cDNA and reconstructing the 5’-UTR and 3’-UTR through 5’ and 3’RACE-PCR. The A and B genome loci were easily discriminated by differences in the nucleotide sequence.

Using the reconstructed sequences it will be possible to design primers able to amplify the whole gene sequence, identify different alleles, and determine the association of allelic variants with phenotypic variation for endosperm colour, in segregant populations and collections of wheat cultivars.
FUNCTIONAL MARKERS FOR GLUTAMINE SYNTHETASE AND CORRELATION WITH GRAIN PROTEIN CONTENT IN DURUM WHEAT

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functional markers, glutamine synthetase, candidate gene, wheat

Durum wheat (Triticum turgidum L. var. durum) is one of the most important cereal crops grown world-wide and provides most of the proteins in human diet, especially in the less developed countries. Seed storage proteins are directly related to the nutritional and technological value of the derived products. Several studies have attested the key-role of the glutamine synthetase enzyme in plant nitrogen metabolism. Glutamine synthetase gene encodes for an enzyme responsible of the first step of ammonium assimilation and transformation into glutamine and glutamate, essential compounds in amino acid-biosynthetic pathway. High protein content is a very important quantitative trait controlled by several genes located on wheat chromosomes. Glutamine synthetase genes are located on the homeologous chromosomes 2A, 2B, and 2D where several authors reported major QTL for protein content. The goal of the present study was to assess the linkage between GS gene and the QTL for protein content. For this purpose, the nucleotide sequence of glutamine synthetase gene acc. DQ124214 was aligned to all the wheat ESTs available in public data bases by means of BLAST tool (http://www.wheat.pw.usda.gov/GG2/blast.shtml). The bioinformatic analysis allowed to find 40 sequences with a similarity > 94% to the GS2 gene, of which three covered the whole gene sequence (DQ124213, DQ124212 and CJ705909). For each of these sequences we designed two or three primer pairs identifying a total of 7 functional markers that were screened among the parents of three segregant populations. Mapping analysis performed by Join Map software allowed to localize the amplified polymorphic fragments and to identify 4 loci: Gs-A2, Gs-B2, Gs-A4, Gs-B4, respectively mapped on chromosome 2A, 2B, 4A and 4B. The QTL analysis for protein content was carried out in a RIL population obtained from the crossing the two durum wheat cultivars Cicco and Svevo. Two major QTLs were identified through Composite Interval Mapping (CIM) performed by the Q-Gene software: one QTL was identified by the functional marker Gs-B2 located on chromosome 2B, and the other one was identified by the functional marker Gs-A4 located on chromosome 4A. These data were confirmed by a linkage disequilibrium analysis carried on a collection of 75 different wheat genotypes.

The present study represents the first step for the identification and sequencing of GS2 gene, which could be employed in breeding programs aimed to increase grain protein content commercial cultivars. Moreover, Gs-B2 and Gs-A4 represents functional markers that could be also efficiently used in marker assisted selection (MAS) programs and map-based cloning.
DETECTION OF QTLs FOR GRAIN YIELD COMPONENTS IN DURUM WHEAT UNDER WATER STRESS CONDITIONS

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durum wheat, QTLs, grain yield, water stress

Water stress is one of the most important abiotic stress that adversely affects wheat production in many regions of the world. Knowledge about number, genomic localization, and quantitative traits loci (QTLs) effect of yield components would facilitate marker-assisted selection for the development of cultivars characterized by high grain yield under water deficit.

The goal of this study was the identification of QTLs controlling grain yield and its components under different moisture regime.

A set of 120 recombinant inbred lines (RILs) was developed from a cross between two durum wheat cultivars (Svevo and Ciccio) by single-seed descent. The parental lines and the RILs were evaluated under rainfed and nonstressed treatments for grain and yield components in two locations of Southern Italy for three seasons. This study was based on a previous Svevo x Ciccio genetic map composed by thirty-eight linkage groups, consisting of 122 EST-SSR, 166 gSSR and 586 DArT markers. The QTL analysis was performed by inclusive composite interval mapping (ICIM) procedure. The threshold to score the presence of a significant QTL for each trait-moisture regime combination was defined by 1000 permutations at P≤0.05.

A total of 18 different QTLs were detected for grain yield, 15 and 10 of which were respectively found under nonstressed and rainfed conditions. This result suggests that more QTLs can be detected in nonstressed conditions since the environmental variability is lower in comparison to that recorded under drought conditions. A major QTL for grain yield was detected on chromosome 3BS (QYld-3BS) in both treatments and in all locations for the three seasons confirming its stability in different environments. The QYld-3BS showed additive effects and R² values ranging from 0.11 to 0.27 t ha⁻¹ and from 10.0 to 34.5% respectively. The alleles present in Ciccio increased grain yield. Moreover, a QTL for 1000-grain weight was also localized on chromosome 3BS indicating that the same QTL was involved in phenotypic expression of the two considered traits. Also for yield per spike and 1000-grain weight, the highest number of QTLs was detected in nonstressed conditions.

Several QTLs for yield components were detected in one treatment or one location. This confirms the significant genotype x treatment and genotype x environment interactions identified in the field trials. Therefore, the evaluation of yield components and the detection of stable QTLs should be ideally, performed in suitable environments.
OFANTO X CAPPELLI, AN INTEGRATED DART-SSR LINKAGE MAP OF DURUM WHEAT FOR DISSECTION OF TRAITS LINKED TO GRAIN YIELD AND WATER DEFICIT TOLERANCE


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genetic map, durum wheat, stress tolerance

Durum wheat (Triticum turgidum L. var. durum) is largely grown in Mediterranean environments where drought stress affects grain yield and yield stability. Drought tolerance, high yield and yield stability are key agronomic traits characterized by a complex genetic basis, being controlled by many loci throughout the genome. This work aimed to develop a new durum wheat intervarietal genetic map based on SSR and DArT markers for the dissection of the genetic bases of important agronomic traits. 161 recombinant inbred lines (RILs) F8-F9 derived from the cross between durum wheat varieties Ofanto and Cappelli were used in this study. Ofanto is a modern cultivar with yield capacity and stability; Cappelli is an old cultivar with lower yield but higher water use efficiency (WUE) with respect to Ofanto. The genetic map comprises 132 SSR, 4 TRAP and 439 DArT markers distributed within 21 linkage groups. A significant deviation from the expected mendelian ratio was registered in segregation for 13.8% of the markers. 42.9% of markers were localized on the A genome chromosomes, while 57.1% were distributed on the B genome chromosomes. The A genome accounted for a map length of 608 cM, while the B genome for 793.6 cM. The employment of the map in the dissection of physiological traits related to water stress tolerance is presented.
CRESO X PEDROSO, A NEW INTEGRATED DART-SSR LINKAGE MAP FOR DISSECTION OF AGRONOMIC TRAITS IN DURUM WHEAT


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linkage map, durum wheat, resistance gene, leaf rust

The construction of genetic maps based on molecular markers represents the first step for the dissection of genetic basis of complex traits and for the identification of closely associated molecular markers useful to transfer the favourable alleles into elite cultivars by MAS programs.

A new genetic map of durum wheat is presented in this work, based on a segregating population derived from the cross Creso x Pedroso and consisting of 123 RILs. A total of 500 molecular markers (173 PCR-based and 327 DArT markers) were assigned to 21 linkage groups. All chromosomes were represented; for 9 chromosomes a single linkage group was identified, while the remaining five chromosomes were associated to two or three linkage groups. Globally, each chromosome was covered by a number of markers ranging from 10 to 62. The final map length was more than 1,800 cM, with chromosome 7B having the greatest coverage, with 62 markers (about 160 cM). Information on the map position of about 100 DArT markers, with unknown location, was provided, contributing towards making these markers a valuable tool for construction and comparison of genetic maps in durum wheat. The map was used to map a major gene accounting for both hypersensitivity response and partial resistance to leaf rust in Creso (Lr14c on chromosome 7BL); furthermore it represents a valuable tool to dissect the genetic basis of other traits of agronomic relevance as root and leaf development in early growing stages.
FINE MAPPING OF TWO MAJOR QTL FOR YIELD IN DURUM WHEAT LOCATED IN THE DISTAL REGIONS OF CHROMOSOMES 2BL AND 3BS


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durum wheat, Quantitative Trait Loci (QTL), grain yield, fine mapping

Most of the durum wheat (Triticum durum Desf.) growing areas in Mediterranean environments suffers from water scarcity and erratic rainfall patterns. Identification of major quantitative trait loci (QTL) for yield across a broad range of environments offers the opportunity to deploy marker-assisted selection to improve yield and yield stability of crops.

In the EU-funded project IDuWUE, 249 RILs (Kofa x Svevo) were evaluated in 16 trials characterized by a broad range of water availability (rainfed and irrigated) and yield potential (from 0.5 to 5.8 t/ha). Two major, epistatic QTL on chrs. 2BL and 3BS influenced grain yield and related morpho-physiological traits, but not heading date. In both cases, coincidence between the QTL for grain yield and those for plant height, peduncle length, SPAD and thousand kernel weight was observed (Maccaferri et al., 2008, Genetics 178: 489-511).

In the FP7 EU-funded project TriticeaeGenome, near-isogenic lines for the chr. 3BS QTL are being derived in order to proceed with the fine mapping of the QTLs. In this regard, a set of 14 pairs of heterogeneous inbred families (HIFs) with the two contrasting parental haplotypes at the 3BS confidence interval have been created to obtain near isogenic lines and to better characterize the physiological basis of the QTL effects.

The 3BS QTL was originally assigned to a 6 cM interval flanked by Xgwm1034 and Xgwm493 genomic SSRs. A total of ca. 140 sequence-derived SSRs and 80 ISBP markers obtained from BAC end-sequences generated during the construction of the 3B physical map (Paux et al., 2008, Science, 322: 101-104) were screened to add new markers to the 3BS QTL interval. Sixteen new BAC-anchored SSR markers (Cft series) were mapped while nine ISBP were found polymorphic among parental lines and will be screened on the RILs. The QTL analysis carried out with the phenotypic data generated in the IDuWUE project allowed us to better position both QTLs and to localize the chr. 3BS QTL peak in respect of the chr. 3BS physical map framework.

For the QTL in the chr. 2BL region, more refined mapping is being conducted with genomic SSR markers and Conserved Ortholog Set (COS) marker. The QTL cluster maps in a 19 cM interval flanked by Xwmc361 and Xgwm1027. Fourty genomic WMS SSRs and 19 COS marker derived from the synteny between wheat and rice/sorghum/maize/Brachypodium genomes have been screened for polymorphism and respectively nine and one markers have been added to the local map. HIFs will be derived also for this QTL. Up to now, the two QTL regions have been marker-enriched at a resolution of ca. 1 cM.
QTLs FOR YIELD AND YIELD COMPONENTS IN BREAD WHEAT UNDER DROUGHT STRESS

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wheat, Drought, Markers, QTL

Drought is one of the major environmental threats to food security in different regions of the world. Therefore, progress in breeding for drought tolerance is extremely important. The limited number of drought tolerant lines and the non-availability of reliable markers for drought tolerance selection still hamper breeding efforts. Understanding the nature of high grain potential and enhanced yield stability, especially in stress environment, will provide opportunities to improve the breeding process. The first necessary step is the QTL mapping of agronomic traits linked to drought tolerance. An inter-varietal mapping population (RILs) developed from a cross between 'Sardari' (a drought tolerant landrace) and 'Arrehane' (a drought susceptible cultivar) was used for the identification of QTLs controlling agronomic and physiological traits under drought stress regime for two-seasons at ICARDA, Aleppo, Syria. To identify genomic regions for traits contributing to drought tolerance, 920 genomic micro-satellite markers covering the entire genome were screened for polymorphism between parents. Out of these, 265 (29 %) simple sequence repeat (SSR) markers were polymorphic including 52 non-genome specific markers, which were not included in further analyses. The 160 polymorphic markers were then taken to do genotyping among the 114 individuals of RIL F6 using Applied Biosystem 3130xl fragment analyzer. The allele calls were made and mined using Genemapper v3.7 software. Mapmaker 3.0 was used to map the polymorphic markers, while Carthagene V was used to check the efficiency and accuracy of the map produced by the Mapmaker 3.0. Using Mapchart the picture representing the marker location was produced. QTL analysis is underway, and the preliminary results are presented.
PRODUCTION OF A MULTIPARENTAL RIL POPULATION FOR HIGH-RESOLUTION MAPPING IN MAIZE

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maize, multi-parental population, QTL

The development of advanced cross designs that might boost the power of detection of the genetic bases of complex traits remain a crucial goal of modern plant genetics. Here we describe the program currently ongoing for the development of an innovative advanced Recombinant Inbred Lines population, aimed at the genetic and molecular dissection of complex traits in maize. Eight maize inbred lines were selected to include a wide genetic variability for the expression of complex phenotypes and crossed according to a half-diallel design. This genetic material was used as the starting point for producing an extended (>2000) maize 8-ways RIL (8W-RIL) to be used for high-resolution QTL mapping. According to what estimated by The Complex Trait Consortium, which first proposed this model for mouse, such a material should allow mapping QTL with effect size >5% of the total variance to an interval of 0.5 cM using fewer than 1000 lines.

Twenty-eight F1 hybrids from the 8x8 half-diallel were crossed so that only crosses between entries with no parents in common (e.g. cross AB x CD) were allowed (but not AB x AD or AB x BF, etc.). Such obtained 4-waves hybrids (210) were bulked in 70 pools, each composed by all the three 4-waves hybrids bearing the same alleles in all possible parent-of-origin cis combinations (e.g. “ABCD” pool included ABxCD, ACxBD and ADxBC 4-waves hybrids). 8-waves hybrids were then produced by crossing complementary 4-waves hybrids pools (e.g. ABCD x EFGH, CDFG x ABEH, etc.).

The production of 8W-RIL by single-seed descent is currently at the third selfing generation (8W-RIL F3). Performing two generations of selfing per year, we expect to obtain a 8W-RI F6 mapping population by the end of 2010, whereas the molecular characterization of parental lines is scheduled to start in the fall of 2009. All 2-ways hybrids, 4-ways hybrids and 8-ways highly-recombinant hybrids plus the parental inbreds will also be available for phenotypic evaluation of complex traits, including heterotic traits.
DEVELOPMENTAL STABILITY MODULATED BY HSP90 CAN HAVE A ROLE IN ACHIEVING HYBRID VIGOUR

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Plastic response to environmental variation is an important factor for the achievement of a robust phenotype, which can respond appropriately to environmental stimuli. The maintenance of adaptive homeostasis, corresponding to a developmental stability, is a key feature at the basis of heterosis, being known that hybrids tend to be more stable than parents. It has been suggested that chaperone molecules such as Hsp90 may play a role in either sensing or integrating environmental signals into the appropriate responses. Indeed, Hsp90 proved to chaperone the signalling proteins that control plant growth and development and so it has been proposed to play a central role in the achievement of adaptive homeostasis. Moreover, the presence of cryptic polymorphism, whose expression depends on Hsp90 activity, has wide implications for phenotypic robustness in Arabidopsis (Queitsch et al., 2002, Sangster et al. 2004, 2008) and has also been proposed to be involved in determining heterosis.

In a study conducted by Frascaroli et al. (2007), the control of heterosis in maize (Zea mays L.) was investigated combining both classical and molecular methods and several quantitative trait loci (QTL) involved in heterosis were identified. A subsequent analysis in the same genetic material (Cane' et al., 2005) revealed co-location of QTL for heterosis and QTL for reaction to a Hsp90 specific inhibitor (geldanamycin, GDA) able to reveal silent genetic variation.

Objective of this study was to identify QTL at the basis of developmental stability in the same maize mapping population and to verify their possible correspondence with the QTL controlling heterosis.

The mapping population consisted of 142 recombinant inbred lines (RILs) of the cross B73 x H99, characterized for more than 200 SSRs and AFLPs markers (Pea et al. 2009). RILs were crossed to both parental inbreds to obtain two testcrosses (TCs) for each RIL. Sterilized seeds of all TCs were soaked overnight without (control, C) or with GDA (treated, T). Two replications were considered. For each replication and treatment, 15 soaked seeds were then transferred to a Petri dish and incubated on filter paper at 20 °C in the dark for 21 days. Shoot (coleoptile) length, root length and a score of root abnormalities (curled, twined roots and abnormal hair production) were assessed in C and T conditions on each individual seedling. Developmental stability for investigated traits was evaluated as Levene's statistics (Shultz, 1985) among single seedling measurements within each Petri dish.

QTL controlling developmental stability were identified in C and T conditions by adopting a model allowing the estimation of additive and dominance effects. Fewer QTL were detected in C rather than in T conditions. Some of the QTL found in T mapped at the same positions as QTL for reaction to GDA. Interestingly, most of the QTL for developmental stability detected in T, i.e., when Hsp90 was impaired by the treatment, co-located with QTL controlling heterosis for grain
yield and other agronomic traits. These findings suggest that adaptive homeostasis, as modulated by Hsp90, has a genetic control and can actually have a role in achieving hybrid vigour.
QTL MAPPING FOR ROOT ARCHITECTURE AT THE SEEDLING STAGE IN MAIZE


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Zea mays, root traits, Quantitative trait loci (QTL), introgression library(IL)

The root system architecture is a complex trait involved in the plant's ability to acquire water and nutrients from the soil as well as in root-lodging resistance. In order to elucidate the genetic control of root architecture in maize, an introgression library (IL) developed from the cross between two parents contrasting for root traits (B73 and Gaspé Flint) was studied. The IL collection included 75 lines, most of which retaining one single chromosome introgression of the donor genome (Gaspé Flint) with an average length of ca. 40 cM. It has been estimated that ca. 70% of the Gaspé Flint genome is represented within the collection.

The IL lines were evaluated for root characteristics by applying two different methodologies, i.e. a paper-roll based protocol and a pot-growing system (seedlings grown until the fourth-leaf stage in sand/clay pebble pots). Root traits (e.g. length and dry weight of the primary and seminal roots, number of seminal roots) and seedling traits (dry weight, etc) at the seminal and early stages of development were recorded. Highly significant differences were observed between the two parental lines and among the IL lines for the number of seminal roots developing from the scutellar node. B73 produced an average of 2.8 seminal roots per plant while Gaspé Flint did not show any seminal root. Among the IL lines, a few showed a Gaspé-like phenotype, implying that the QTLs controlling this trait are localized on the corresponding introgressions. Five QTLs for seminal root number were identified in the paper roll experiment, three of which were also confirmed in the pot experiment.

In order to better characterize the QTL on chromosome 1 (Seminal root 1, Sr1), F2 plants from the cross between B73 and the IL line carrying the introgression on chromosome 1 have been analyzed for both phenotype, using the paper roll system, and genotype using SSRs mapped in the introgressed region. A major QTL for seminal root number has been confirmed on chromosome 1 bin 1.02; the QTL is characterized by a LOD value equal to 16, spans ca. 7 cM and is localized in the proximity of locus Rtcs1 (Taramino et al., 2007. Plant J. 50: 649-659) controlling root architecture. Thus phenotypic and preliminary fine mapping data indicate that the two loci do not coincide.

Positional cloning of Sr1 is underway.
DEVELOPMENT OF A DArT PLATFORM IN GRAPEVINE AND ITS APPLICATION TO GENETIC RESOURCES EVALUATION

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Diversity Array Technology, Vitis vinifera, biodiversity

The renewed interest for grapevine genetic resources is justified by the fact that natural genetic variation is invaluable for crop improvement and understanding of gene function. Microsatellite markers have been classically used in several studies, with major application directed to the analysis of the genetic structure of grapevine cultivar gene pools, but even to the identification and discrimination of cultivars for collection management and for plant trade. Despite the numerous advantages of these markers, SSR use is limited because of the low number of loci that can be simultaneously analysed per experiment. In our work a DArT (Diversity Array Technology) platform has been developed to assay SNP and InDels that are ‘randomly’ selected across the whole grapevine genome. In fact, both the large number of markers that can be simultaneously assayed and the random nature of DArT markers can provide high level of resolution in genetic-diversity studies and accurate genetic-distance estimates. DArT approach has been applied to evaluate genetic variability in a grapevine cultivar collection including both wide-growing and typical varieties, mainly from Liguria region. Phylogenetic analysis and molecular traceability approaches have been developed starting from the data obtained. The perspectives of this work are directed to the use of this molecular marker class in linkage disequilibrium studies.

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