Development of a multi-parental (four-way cross) mapping population for multi-allelic QTL analysis in durum wheat

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ABSTRACT

Classical bi-parental F_2 mapping progenies do not allow the mapping of multiple alleles and the study of their interactions. DISTA and Società Produttori Sementi have assembled a recombinant inbred mapping population developed from a balanced four-way cross using four cultivars (Neodur, Claudio, Colosseo and Rascon) characterized by different yield and quality parameters and by resistance to powdery mildew, leaf rust and *Fusarium* head blight. A genetic map was generated based on AFLP and chromosome-specific SSR markers using individuals from a single segregating four-way F_1 population. Assembly of the genetic map was performed using a pedigree-based multi-locus mapping software (CRI-MAP). The genetic map will be integrated with SNP markers, newly discovered using the CRoPS™ marker technology recently developed by Keygene N.V. The integrated map will then be used on inbred lines to identify QTLs of important quality and resistance traits in durum wheat. This multi-parental approach will allow for a more efficient analysis of the effect of multiple alleles and their epistatic interactions at single QTLs of complex traits such as yield, quality and response to wheat fungal diseases.

INTRODUCTION

The development and analysis of multiparental inbred populations, using various crossing schemes and methodology of QTL analysis, has been repeatedly proposed as a more efficient alternative to the classical development of large bi-parental populations to unravel the complex pattern of epistatic QTL interactions. Among the proposed approaches are the use of interconnected populations (using di-allelic schemes or common "pivotal" parents, Blanc et al., 2006) and the development of balanced recombinant inbred populations from multiple founders / heterogeneous stocks (Flint et al., 2005; Broman 2005). These populations, if correctly developed, should bypass the population structure-associated QTL mapping constraint, which is a major problem of the association mapping approach in crop germplasm collections (Yu et al., 2006). In particular, wheat germplasm collections show large evidence of population structure (Maccaferri et al., 2005). The durum wheat elite germplasm carries useful genetic variation for resistance to the most common wheat pathogens, quality traits, yield, and yield stability but, with only a few exceptions (Nachit et al., 2001; Maccaferri et al., 2008), until now little progress has been achieved to map the determinants of this genetic variation. To this end, four highly diverse durum cultivars were selected as representatives of important durum "breeding lineages" widely exploited in the Italian breeding programs to develop a four-way mapping population.

MATERIALS AND METHODS

Plant material and crossing design

A balanced four-way multi-parental cross was developed by DISTA and Società Produttori Sementi using four durum wheat cultivars (Neodur, Claudio, Colosseo and Rascon) carrying *Fusarium*, leaf rust and powdery mildew resistance genes and different yield and quality traits. The four-way F_1 population is being genotyped for genetic map development, while the final RIL population (ca. 380 F_8 RILs) will be available by the end of 2008.

Genetic map development

A total of 90 four-way F_1 samples were genotyped with AFLP and SSR markers. AFLP were generated following the protocol of Vos et al. (1995). Different restriction enzymes combinations (*Eco*RI/*Mse*I, *PstI*/*Mse*I, *Eco*RI/*Taq*I and *Pst*I/*Taq*I) and number of selective nucleotides were tested to optimize the AFLP technique on durum wheat; of these, *Pst*I+3/*Taq*I+3 was selected as it provided the highest quality and number of detectable amplicons. In order to determine the most informative primer combinations (PC) a pre-screening on the four parental lines was performed using 101 PC. The most polymorphic 25 PCs were selected for genotyping the F_1 samples. AFLP markers were integrated with additional 90 chromosome-specific SSR marker profiles generated using the LI-COR 4200 IR2 System. Due to the complexity of the parental crossing scheme, the construction of the genetic map was performed using CRI-MAP program, a pedigree-based multilocus mapping software (Lander and Green, 1987).

RESULTS AND DISCUSSION

Genetic map

Among the 216 AFLP markers obtained from 25 PCs and the 90 SSR markers, a total of 259 markers (189 AFLP and 70 SSR) were mapped on 22 linkage groups, spanning a total of 1080 cM. Based on the information of the chromosome-specific SSR markers, the 22 linkage groups partially cover 13 of the 14 durum chromosomes. Based on these preliminary results proving only a partial coverage of the durum genome, it was decided to further integrate the map with single nucleotide polymorphism

(SNP) markers. Keygene N.V. has recently developed the complexity reduction of polymorphic sequences (CRoPS™) technology as a novel approach for SNP discovery in plants (van Orsouw et al., 2007). CRoPS is based on the detection of polymorphisms between sequences of two or more genetically diverse samples by combining the power of reproducible genome complexity reduction of AFLP with the novel sequencing-by-synthesis (pyrosequencing) technology of Roche's Genome Sequencer FLX. Briefly, sample DNA is reduced in complexity by AFLP and sequenced at more than 5-fold redundancy in high-density picolitre reactions. Reads are processed and analyzed using a custom-build bioinformatics pipeline and mined for SNP. CRoPS analysis with the four parental lines (589,826 sequences with an average length of 155 bp), evidenced more than a thousand putative SNP between lines. A subset of these putative SNP will be used to genotype the 90 four-way F_1 samples using SNPWave (30- to 90-plex; van Eijk et al. 2004) and VeraCode-BeadXpress (96- to 384-plex) genotyping assays. The integration of the current AFLP/SSR-based map with further SNP markers will improve the quality and genomic coverage of the genetic map. Future research will focus on phenotyping and genotyping RIL samples for QTL analysis. With respect to the use of traditional bi-parental mapping populations, this multi-parental approach will allow for a more efficient analysis of the effect of multiple alleles and of their epistatic interactions at loci influencing important traits in durum wheat.

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REFERENCES

Blanc G, Charcosset A, Mangin B, Gallais A, Moreau L (2006). Connected populations for detecting quantitative trait loci and testing for epistasis: an application in maize. Theor Appl Genet, 113: 206-224

Broman KW. (2005) The genomes of recombinant inbred lines. Genetics, 169: 1133-1146

Flint J, Valdar W, Shifman S, Mott R. (2005). Strategies for mapping and cloning quantitative trait genes in rodents. Nat Rev Genet, 6: 271- 286

Lander ES and Green P (1987) Construction of multilocus genetic linkage maps in humans Proc of Natl Acad Sci (USA), 84: 2363-2367

Maccaferri M, Sanguineti MC, Noli E, Tuberosa R (2005). Population structure and long-range linkage disequilibrium in a durum wheat elite collection. Mol Breed, 15: 271-290

Maccaferri M, Sanguineti MC, Corneti S, Araus Ortega JL, Ben Salem M, Bort J, DeAmbrogio E, Garcia del Moral L, Demontis A, El-Ahmed A, Motawaj J, Maalouf F, Machlab H, Martos V, Nachit M, Nserallah N, Ouabbou H, Royo C, Slama A, Moragues M, Tuberosa R (2008). Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability. Genetics 178: 489-511.

Nachit MM, Elouafi I, Pagnotta MA, El Saleh A, Iacono E, Labhilili M, Asbati A, Azrak M, Hazzam H, Benscher D, Khairallah M, Ribaut JM, Tanzarella OA, Porceddu E, Sorrells ME (2001). Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (Triticum turgidum L. var. durum). Theor Appl Genet 102: 177-186.

van Eijk MJ, Broekhof JL, van der Poel HJ, Hogers RC, Schneiders H, Kamerbeek J, Verstege E, van Aart JW, Geerlings H, Buntjer JB, van Oeveren AJ, Vos P. (2004). SNPWave: a flexible multiplexed SNP genotyping technology. Nucleic Acids Research, 32(4): e47

van Orsouw NJ, Hogers RC, Janssen A, Yalcin F, Snoeijers S, Verstege E, Schneiders H, van der Poel H, van Oeveren J, Verstegen H, van Eijk MJ. (2007). Complexity Reduction of Polymorphic Sequences (CRoPS™): A Novel Approach for Large-Scale Polymorphism Discovery in Complex Genomes. PLoS ONE, 2: e1172

Vos P., Hogers RC, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research, 23: 4407-4414

Yu J, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Holland JB, Kresovich, S, and Buckler, ES (2006). A unified mixed-model method for association mapping accounting for multiple levels of relatedness. Nature Genetics, 38: 203-208