Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 6A.11

SNP DEVELOPMENT AND VALIDATION IN DURUM WHEAT USING NEXT GENERATION SEQUENCING (NGS) TECHNIQUES

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Single nucleotide polymorphisms (SNP), Triticum durum Desf., Next Generation Sequencing (NGS), linkage mapping, Illumina assay

In durum wheat (*Triticum durum* Desf.), an allotetraploid species (AABB genomes), the development of SNP assays is complicated by the presence of two highly similar homeologous copies for each gene, ancestral paralogous and highly repetitive sequences. Collectively, the combination of low nucleotide diversity, polyploidy and repetitiveness severely limit advances in SNP discovery and genotyping platform development. We describe the application of Complexity Reduction of Polymorphic Sequences (CRoPS®) technology coupled with the Illumina Golden Gate (GG) genotyping assay and of Genotyping by Sequencing for SNP identification and genotyping in the elite durum wheat germplasm.

Starting from the genomic DNA of four diverse durum wheat genotypes (Neodur, Colosseo, Claudio and Rascon), SNP discovery was performed following the CRoPS protocol (van Orsouw et al. 2007. PLoS One 2:e1172) which included a preliminary step of genome complexity reduction based on the AFLP technique and a massively parallel sequencing of the libraries. The tagged libraries from the four genotypes were sequenced in one single run of GS FLX 454 Roche sequencer. A total of 2,659 SNPs were identified on 1,206 consensus sequences. Among the 768 SNPs that were randomly chosen irrespective of their genomic repetitiveness level to be assayed on the Illumina BeadExpress genotyping system, 275 (35.8%) SNPs passed the validation phase. Of these SNPs, 157 were mapped in the biparental mapping populations Colosseo x Lloyd (Mantovani et al. 2008. Mol Breed 22:629–648) or Meridiano x Claudio (Maccaferri et al. 2011. TAG DOI 10.1007/s00122-011-1605-9) for which SSR- and DArT-based framework maps were available. The Illumina genotyping assay of the RILs was carried out on pre-amplified templates to achieve the same level of genomic complexity reduction (*Pst*I + A/*Taq*I + CT) used during the SNP discovery phase. Considering the non-repetitive sequences only, the proportion of correctly genotyped SNPs increased to 47.0% (Trebbi D. et al. 2011. Theor Appl Genet DOI 10.1007/s00122-011-1607-7).

In a second NGS experiment, Genotyping by Sequencing (GBS; Elshire et al. 2011. PLoS One. 6:e19379) was used for the first time in *Triticum durum* to identify and genotype SNPs at the same time. Illumina highly parallel sequencing technology was used to sequence and genotype reduced representation libraries from 91 RILs of the Colosseo x Lloyd mapping population. The sequencing experiment was conducted with pools of 14 tagged genotypes per each Illumina sequencing flow-cell. This allowed to generate and map with high confidence ca. 1,000 high quality

SNPs with less than 10% overall missing data, i.e. a subset of all the SNP information generated with the Illumina sequencing experiment. Our study contributes towards a more cost-efficient and high-throughput whole-genome mapping in wheat.