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

Poster Communication Abstract – 7.12

DE NOVO SEQUENCING OF *ANEMONE CORONARIA* TRANSCRIPTOME TO DISCOVER PUTATIVE GENES INVOLVED IN *TRANZECHELIA DISCOLOUR* INFECTION RESPONCE

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454 sequencing, Gene Ontology classes, KEGG pathways, Prunus/Anemone rust, R-genes

The genus *Anemone* (*Ranunculaceae*) includes many species that are cultivated as ornamentals, either as garden or as cut-flowers plants. The poppy anemone *A. coronaria* is the most valuable winter-flowering species (Yonash *et al*., 2004) and is the progenitor of most varieties currently grown for cut-flower production.

The *Prunus*/*Anemone* rust, caused by the fungus *Tranzschelia discolor*, has become aggressive in *A. coronaria* cultivation in recent years. Teliospores, formed on *Prunus* leaves, infects *A. coronaria* seedling during the growth cycle required to produced rhizomes; infected plants remain asymptomatic. The disease appears in the next vegetative cycle, on plants cultivated to crop flowers. *T. discolour* infection dramatically reduces flower production and quality. Aeciospores released from *A. coronaria* leaves only infect *Prunus*.

Over the few last years, next-generation sequencing (NGS) technologies have led to revolution in genomics and genetics and provided cheaper and faster delivery of sequencing information (Morozova et al., 2008; Mardis 2008). To date, 454 pyrosequencing technology is widely used for *de novo* sequencing and analysis of trascriptomes.

Total RNA was isolated from leaves of *A. coronaria* either healthy or infected by *T. discolor*. Two 3'-fragment cDNA libraries (labeled S_1S and S_2I) were prepared for pyrosequencing with the GS FLX 454 Titanium system (by Eurofins MWG-Operon; Ebersberg, Germany).

454 reads from the two libraries were independently assembled using the MIRA Assembler. The 304.786 and 306.439 HQ reads obtained with an half-plate run from the S_1S and S_2I libraries were assembled in 154.039 and 150.421 unigenes (contigs plus singletons), respectively. The unigene sequences were annotated by BLASTx versus the UniProtKB database as well as the *Puccinia graminis tritici* and *Puccinia triticina* database (http://www.broadinstitute.org/annotation/genome/puccinia group/multiDownload.html). Cross-library comparison were performed with BLASTn to detect library-specific transcripts. A functional classification of the unigenes according to Gene Ontology (GO) was performed with a custom tool and statistical analyses were performed to highlight differentially expressed GO classes within a plant-specific GO-Slim. UniProt-ID retrieved by BLAST were used to relate transcripts to know

molecular pathways available at KEGG. Comparison of unigenes to a plant resistance gene database (PRGdb; http://www.prgdb.org), was also performed with Blast2GO (www.blast2go.org).

The overall analyses allowed the individuation of ontology classes displaying statistically significant differences in expression levels between the two libraries. In addition, 611 and 524 sequences from 1S and 2I library, respectively, presented homology with R-genes collected in PRGdb.