Proceedings of the 52nd Italian Society of Agricultural Genetics Annual Congress
Padova, Italy – 14/17 September, 2008
ISBN **978-88-900622-8-5**

Poster Abstract – D.10

ANALYSIS OF CLONAL VARIATION IN GRAPE CULTIVARS

CATTONARO F.*, BRINDISI A.*, FELICE N.**, SWAMINATHAN S.***,
DI GASPERO G.*,**, POLICRITI A.*,***, MORGANTE M.*,**

*) Istituto di Genomica Applicata, Via Linussio 51, 33100 Udine (Italy)
**) Dipartimento di Scienze Agrarie e Ambientali, Università di Udine, Via delle Scienze 208, 33100 Udine (Italy)
***) Dipartimento di Matematica e Informatica, Università degli Studi di Udine, Via delle Scienze 208, 33100 Udine (Italy)

grape clones, somatic variation, re-sequencing, next generation (re)sequencing, comparative genome hybridization

Grapevine is a vegetatively propagated fruit crop. Many grape varieties propagated by grafting since centuries undergo natural mutations (base mutations, indels, transposable element insertions or excisions, and epigenetic modifications) that can lead to the appearance of desirable phenotypic variations. These genetic variants are selected and commercialized as a new clone of a certain cultivar by nurseries, but up to now there is no reliable tool to discriminate clones from one another and clones can not be patented and protected from illegal multiplication.

The grape material that IGA is analyzing is composed of 3 pairs of clones belonging to 3 different varieties plus 4 clones for the Tocai variety: (1) Pinot noir VCR18 clone vs Pinot Blanc VCR5; (2) Sauvignon R3 clone vs Sauvignon french clone 297; (3) Sangiovese VCR5 clone, biotipo toscano vs Sangiovese VCR23 clone, biotipo romagnolo; (4) Tocai R5 clone, Tocai R14 clone , Tocai French clone and Sauvignonasse.

In the starting phase of the project, eight clones (the first 3 pairs and two Tocai clones, R5 and R14) were genotyped at 180 microsatellite loci scattered across all 19 chromosomes starting from DNA extracted from apical leaves and berry skins. The AFLP (Amplified Fragment Length Polymorphisms) technique was used in parallel to SSR analysis to discover single base polymorphisms due to mismatches or small indels. The two approaches with standard molecular markers provided a reduced amount of information about clonal variation. Then a re-sequencing effort using standard Sanger sequencing has been initiated for Pinot blanc cultivar. More than 41.000 mate-paired reads were produced, correspondent to about 28 Mbp of Pinot blanc genome (0,06x coverage). Mate-paired reads are currently being aligned to the PN40024 genome (Nature, 27 August 2007) and to the Pinot noir ENTAV genome (PloS One, 19 December 2007) to detect the presence of nucleotide and structural variation between Pinot clones.

With the availability of next generation sequencing technologies and the accessibility at low costs of other whole genome scale approaches (i.e. whole-genome scanning microarrays), IGA has started the analysis of grape clones genomes by two approaches: (1) Ultra high-throughput sequencing of reduced representation libraries by the Illumina Genome Analyzer GAII; (2) Microarray based Comparative Genomic Hybridization (CGH) to detect insertion/deletions and DNA copy number differences between couple of clones belonging to the same variety.

The results of such genome wide analyses of grape clones will be transferred to a high throughput, highly automated protocol for the routine analysis of clonal variants