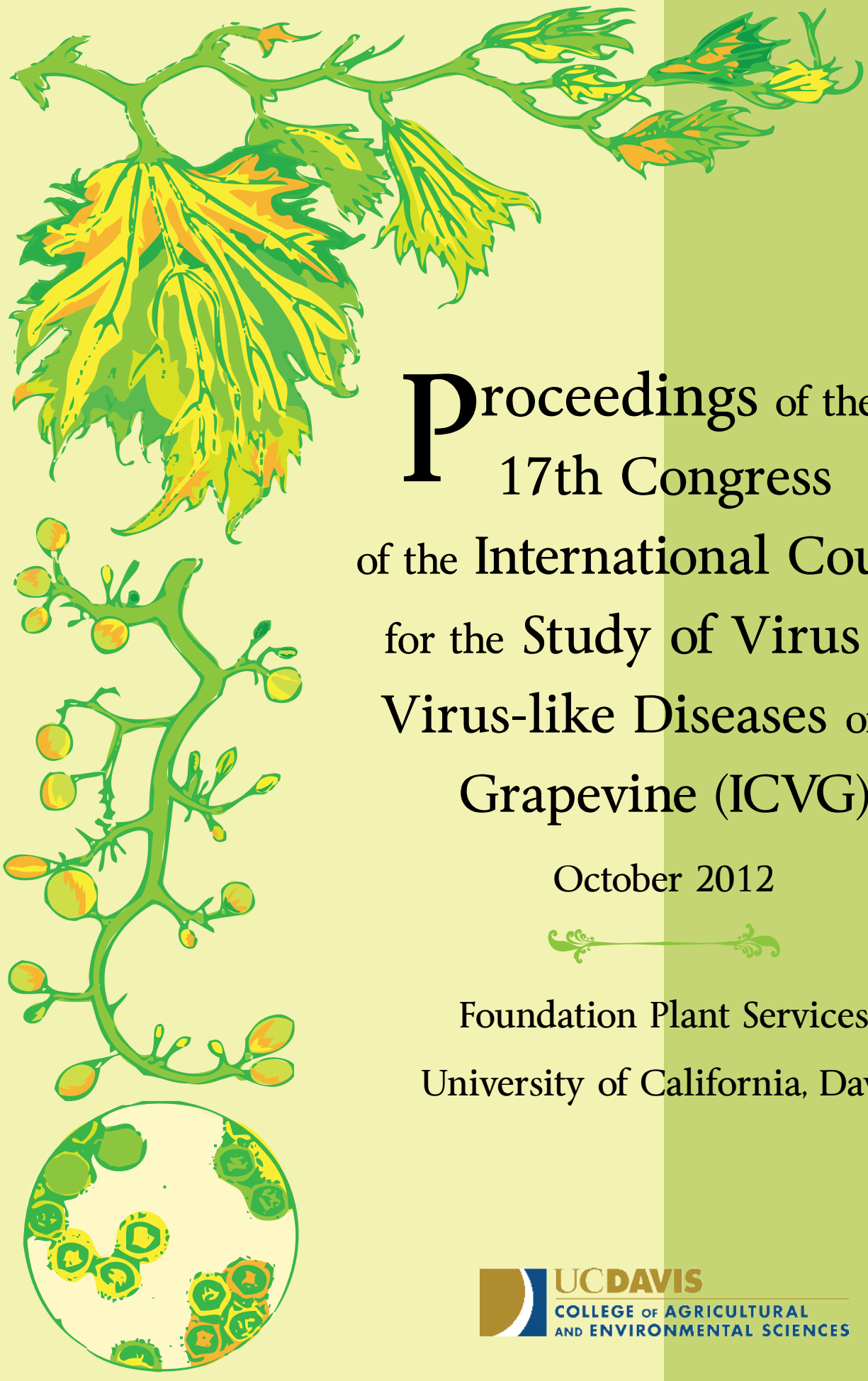

Proceedings of the 17th Congress of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG)

October 2012

Foundation Plant Services
University of California, Davis

UC DAVIS
COLLEGE OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES

Approaches of Next Generation Sequencing to Investigate Grapevine Diseases of Unknown Aetiology

Chiumenti, M.[1], Giampetruzzi, A.[1], Pirolo, C., Morelli, M. , Saldarelli, P.[2] , Minafra, A.[2], , Bottalico, G.[1], La Notte, P.[2], Campanale, A.[2], Savino, V.[1] and Martelli, G.P.[1]

[1]*Dipartimento di Scienze del suolo, della pianta e degli alimenti, Università degli Studi di Bari „Aldo Moro", Via Amendola, 165/A, 70126 Bari, Italy.*
[2]*Istituto di Virologia Vegetale CNR, UOS Bari, Via Amendola, 165/A, 70126 Bari, Italy.*

INTRODUCTION

Analysis of small RNAs (sRNAs) and /or double-stranded RNAs (dsRNA) libraries by next generation sequencing (NGS) is a novel technology that is proving increasingly useful for the discovery of previously unknown viruses associated with diseases of undetermined aetiology (e.g. Loconsole *et al.,* 2012). Among virus-like diseases of the grapevine, enations, an erratic disorder whose appearance may depend on seasonal conditions (Martelli and Boudon Padieu, 2006), is characterized by morphological modifications of the leaves that recall the consequences of hormonal unbalance. Even though graft transmissibility of enation disease supports its viral etiology, the putative agent has not yet been identified. NGS technology was used to investigate the "virome" of enation-infected grapevines and of an accession of cv. Moscato giallo showing an unusual interveinal spring chlorosis, that tends to turn whitish-yellow as the season advances.

MATERIALS AND METHODS

Grapevine sources: Leaves collected in late spring from eight plants of cv. Panse precoce with enations in a 10-year-old commercial vineyard from Trani (Apulia, Southern Italy) and symptomatic leaves of cv. Moscato giallo from a vineyard at Locorotondo (Apulia, Southern Italy) were pooled for NSG.

Libraries preparation and analysis: Purified dsRNAs and small sRNAs from leaf tissues were used to synthesize cDNA libraries according to the Illumina protocol (Giampetruzzi *et al.*, 2012). A 50 base-single read run was done on a HiScan SQ apparatus. Short sequences were processed with Fastx toolkit, *de novo* assembled with Velvet (Zerbino and Birney, 2008) and searched for homologies with viral sequences with BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) tools. Guided assembly of viral genome sequences was with SOAP (http://soap.genomics.org.cn/) using reference sequences of GFLV, *Grapevine leafroll-associated virus 3* (GLRaV-3) and *Grapevine virus A* (GVA), retrieved from the RefSeq database (ftp://ftp.ncbi.nih.gov/refseq/release/viral/).

RESULTS AND DISCUSSION

Enations: Assembly of sequences obtained from the dsRNA library produced 2,040 contigs, whose BLASTN analysis revealed homologies with 10 different viruses: *Grapevine leafroll-associated virus 1, 2, 3, 5 and 9* (GLRaV-1,-2,-3,-5,-9), GVA, *Grapevine virus B* (GVB) *Grapevine rupestris stem-pitting associated virus* (GRSPaV), GFLV and *Grapevine fleck virus* (GFkV). From the small RNAs library 355 contigs were obtained that showed homologies to six viruses: GLRaV-1, GLRaV-2, GVA, GFLV, and GFkV; and two viroids: *Grapevine yellow speckle viroid-1* (GYSVd-1) and *Hop stunt viroid* (HSVd). Comparative analysis of both libraries disclosed the prevailing presence of GLRaVs sequences in the dsRNA library, presumably due to the propensity of these viruses to accumulate RNA replicative intermediates. Conversely, the small RNA library allowed the assembly of a larger number of GFLV contigs probably consequent to its active replication during spring.

Routine virus detection by RT-PCR assays (Table 1) confirmed the NGS-detected viruses and confirmed the occurrence of GLRaV-9 in Italy (Giampetruzzi *et al.*, 2011). A comparative analysis of a group of plants from the same vineyard subjected to sanitation by thermotherapy, which did not show symptoms of enations for over three years (Table 1), showed a less compromised sanitary status although being still infected by GFLV. A similar situation had been encountered in enation-affected vines of cv. Michele Palieri (unpublished data). This data confirms the alleged lack of relationship between GFLV and enation disease but does not clarify the nature of the disease whose aetiology still remains undetermined.

	Healthy	E1	E2	E3	E4	E5	E7	E8	T1	T2	$T2_1$	$T2_2$	$T2_3$
GRSPaV	-	+	+	+	+	+	+	+	+	+	+	+	+
GFLV	-	+	+	+	+	+	+	+	+	+	-	+	+
GLRaV-1	-	+	+	+	+	+	+	+	-	-	-	-	-
GLRaV-2	-	+	+	+	+	+	+	+	-	-	-	-	-
GLRaV-3	-	+	+	+	+	+	+	+	-	-	-	-	-
GLRaV-5	-	-	-	-	-	+	-	-	-	-	-	-	-
GLRaV-9	-	+	+	-	+	+	+	-	+	-	-	-	-
GFkV	-	+	+	+	+	+	+	+	+	+	+	+	+
GVA	-	+	+	+	+	+	+	+	+	-	-	-	-
GVB	-	-	-	-	+	-	-	-	-	-	-	-	-

Table 1. Sanitary status of grapevine plants with enations (E series) assessed by NGS and RT-PCR. A group of partially sanitized plants (T series), originating from the same vineyard and not showing enation symptoms was tested for comparison.

Spring chlorosis: assembly short sequences from the Moscato giallo vine produced 10,198 and 1,708 contigs respectively from the dsRNA and sRNA libraries. Six different viruses and one viroid were identified in the library from dsRNA: GLRaV-2*,* GLRaV-3*,* GVA*,* GRSPaV*,* GFkV and GFLV and GYSVd1 whereas the analysis of the sRNA library showed the presence of GFLV*, Grapevine Red globe virus* (GRGV)*, Grapevine rupestris vein feathering virus* (GRVFV)*,* and two viroids: GSYVd1 and HSVd. GLRaVs , GVA and GRSPaV were not found, the same as in the libraries from enation disease. Also with Moscato giallo, the presence of the detected viruses was confirmed by RT-PCR. The complete RNA sequences of the GFLV isolate from these plants was reassembled and showed a significant amino acid identity of both polypropteins P1 (98,1%) and P2 (97,6%).

The conclusion is that NGS technology gives a very wide if not complete picture of the virome of a given vine (Al Rwahnih *et al*., 2009; Coetzee *et al*., 2010), an that the use of dsRNAs or sRNAs as template for library preparation and the run of samples in a multiplex format is a better option for the identification of the viruses present in the plant under study.

ACKNOWLEDGEMENTS

Work supported by the Project CISIA “Valorizzazione delle risorse genetiche di colture mediterranee attraverso approcci di metagenomica, trascrittomica ed analisi funzionale per la caratterizzazione di germoplasma autoctono, endofiti, agenti di biocontrollo e fitopatogeni”.

REFERENCES

Al Rwahnih, M., Daubert, S., Golino, D., Rowhani, A., 2009. Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus infection that includes a novel virus. Virology 387, 395–401.

Coetzee, B., Freeborough, M.J., Maree, H.J., Celton, J.M., Jasper, D., Rees, G., Burger, J.T., 2010. Deep sequencing analysis of viruses infecting grapevines: virome of a vineyard. Virology 400, 157–163.

Giampetruzzi, A., Chiumenti, M., Roberto, R., Pirolo, C., Minafra, A., Saldarelli, P. 2011. First detection of *Grapevine leafroll–associated virus 9* in Italy. Journal of Plant Pathology (2011), 93 (4, Supplement), S4.63-S4.89.

Giampetruzzi, A., Roumi, V., Roberto, R., Malossini, U., Yoshikawa, N., La Notte, P., Terlizzi, F., Credi, R., Saldarelli, P. 2012. A new grapevine discovered by deep sequencing of virus- and viroid-derived smallRNAs in Cv Pinot gris. Virus Res. 163:262-268.

Loconsole, G., Saldarelli, P., Doddapaneni, H., Savino, V., Martelli, G.P., Saponari, M. 2012. Identification of a single-stranded DNA virus associated with citrus chlorotic dwarf disease, a new member in the family *Geminiviridae*. Virology, 432: 162-172.

Martelli, G.P. and Boudon-Padieu, E. 2006 Directory of Infectious Diseases of Grapevines. Options Méditerranéennes, Série B, 55: CIEHAM-IAM Bari.

Zerbino, D.R., Birney, E., 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 18, 821–829.