

Validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules

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The Italian Ministry of Agriculture has financed the Finalized Project “ARNADIA” with the purpose to produce validated reference diagnostic protocols for the control and monitoring of plant pathogens of phytosanitary interest. The grapevine viruses covered by phytosanitary rules were identified among them. To this end, it has been established the “Working group ARNADIA – grapevine viruses (WG)” which included 8 Research Institutions, 3 accredited Private Laboratories, one Plant Health Service and one Association of Grapevine Nurseries.

The aim of WG was to produce reference and validated serological and molecular protocols allowing for the harmonization of the diagnosis of 8 grapevine viruses (GLRaV 1, 2, 3, GVA, GVB, ArMV, GFLV and GFKV).

A protocol validation is the evaluation of a process to determine its fitness for a particular use. A validated assay yields test results that identify the presence of a specific target. Parameters that influence the capacity of the test result to predict accurately the infection status of the sample are diagnostic sensitivity (ability of the method used to detect the presence of the pathogen in the samples surely infected by the pathogen in



question - true positive) and diagnostic specificity (ability of the method used to not detect the presence of the pathogen in samples not infected by the pathogen in question - true negative). Other parameters that must be considered and which determine the efficiency of a protocol are: the analytical sensitivity (the smallest amount of infectious entities that can be identified by the diagnostic method), repeatability (degree of conformity of the results obtained in replications of the method, made at short intervals of time, using the same reference sample and in the same working conditions i.e. equipment, operator, laboratory) and reproducibility (degree of conformity of the results obtained using the same method with the same reference samples in different laboratories).

In this view, 122 grapevine samples (varieties, rootstocks and pool) have been analyzed by serological (using 24 antisera of three commercial companies) and molecular (multiplex RT-PCR) protocols. Moreover, different extraction methods, reagents and materials have been compared in 13 laboratories. Processing of the obtained results (about 24,000 data) has led to the definition of validation parameters according to UNI/ENI/ISO 16140 and 17025 and EPPO standards PM7/76 and PM7/98.

ELISA has proved to be a highly effective technique comparable with the molecular method, although the latter, as expected, it turned out to be more efficient for some viruses and on specific samples (rootstocks and pool). On these bases, serological and molecular protocols could be considered as alternative methods and their use has been suggested for different specific applications.

All results and parameters obtained will be the subject of detailed discussion.