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Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules

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The Italian Ministry of Agriculture funded in 2009 the "ARNADIA" Project, aimed at producing validated reference diagnostic protocols for the control and monitoring of plant pathogens of phytosanitary interest and, among them, grapevine viruses. In this framework, the "Working group ARNADIA – grapevine viruses (WG)", composed of eight universities and research bodies, three accredited private laboratories, one plant health service and one association of grapevine nurseries, was established.

The aim of the WG was to produce referenced and validated serological and molecular protocols allowing for the harmonization of diagnosis of eight grapevine viruses: *Grapevine leafroll-associated virus* 1, 2, 3, (GLRaV 1, 2, 3) *Grapevine virus* A (GVA), *Grapevine virus* B (GVB), *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV) and *Grapevine fleck virus* (GFkV).

The validation of a protocol consists of the evaluation of specific parameters designed to determine their suitability to identify the presence of a specific "target". The parameters that influence the capability of the test to accurately predict the sample's infection status are the diagnostic sensitivity (ability of the utilized method to detect the presence of the pathogen in the samples truly infected by the pathogen in question - true positive) and diagnostic specificity (ability of the utilized method NOT to 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 or accordance (degree of conformity of the results obtained in replications of the process, made at short time intervals, using the same reference sample and in the same working conditions i.e. equipment, operator, laboratory) and reproducibility or concordance (degree of conformity of the results obtained using the same method with the same reference samples in different laboratories). The latter parameter was defined in collaboration with five laboratories of the Regional Phytosanitary Services.

Specifically, 122 grapevine samples (varieties, rootstocks and "pools" of five plants, of which only one infected) were analyzed by ELISA, using 25 antisera from three commercial companies (Agritest, Bioreba, Sediag) and multiplex RT-PCR protocols. For ELISA, the tests were conducted carefully following instructions provided by the companies; multiplex RT-PCR was performed using the protocol described by Gambino and Gribaudo (2006). The tests were performed in 18 laboratories using the same samples (analyzed in blind conditions) and reagents. In each laboratory, results were obtained using the same threshold value calculated on the basis of the spectrophotometer readings for ELISA and by analyzing the electrophoretic gels for the multiplex RT-PCR. Processing of the obtained results (about 24,000 data points) led to definition of the validation parameters according to UNI/EN/ISO 16140 and 17025 and EPPO standards PM7/76 and PM7/98.

As reported in Table 1, ELISA proved to be a highly effective technique, comparable to the molecular method, although the latter turned out, as expected, to be more efficient for some viruses and on some specific samples (rootstocks and "pool").

Table 1 - Summary of validation parameters obtained by the ELISA test for each virus and antiserum and comparison with those obtained with the molecular protocol

Virus	Diagnostic protocol	Sensitivity	Specificity	Accuracy	Analytical sensitivity	Repeatability	Reproducibility
ArMV	Multiplex	92 %	99 %	98 %	10^{-2}	100%	100 %
	ELISA – A/B/S	64/48/50%	85/95/96%	74/72/72%	10^{-2}	100%	95%
GFLV	Multiplex	68 %	100%	90 %	10^{-3}	100%	76%
	ELISA – A/B/S	75/82/77%	96/92/92%	80/84/81%	10^{-4}	100%	90%
GFkV	Multiplex	95%	95%	95%	10^{-2}	100%	95%
	ELISA – A/B/S	90/90/30%	100%	92/92/46%	10^{-1}	98%	88%
GVA	Multiplex	96 %	99 %	98 %	10^{-2}	100%	94 %
	ELISA – A/B/S	77/45/87%	100/100/96%	83/58/89%	10^{-1}	98%	82%
GVB	Multiplex	100%	100%	100%	10^{-3}	100%	100%
	ELISA – A/B/S	86/nt/nt%	100%	92%	10^{0} (2^{-3})	100%	85%
GLRaV 1	Multiplex	74 %	100 %	94 %	10^{-2}	100%	70 %
	ELISA – A/B/S	89/94/96%	100%	93/96/98%	10^{-2}	100%	92%
GLRaV 2	Multiplex	84%	98%	85%	10^{-2}	95%	83%
	ELISA – A/B/S	86/67/87%	100%	93/96/98%	10^{0} (2^{-3})	93%	84%
GLRaV 3	Multiplex	100 %	93 %	95 %	10^{-3}	100%	100 %
	ELISA – A/B/S	81/90/97%	100%	84/92/97%	10^{-1}	100%	94%

A= Agritest; B= Bioreba; S= Sediag.

In conclusion, harmonized and validated reference diagnostic protocols for grapevine viruses subjected to phytosanitary rules are, for the first time, available. The efficiency and robustness of the protocols have been proven using a large number of samples in a variety of laboratories. On this basis, both serological and molecular protocols resulted valid, and their use could be as a function of different specific applications.

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References

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