Organization of ring tests on diagnostic methods among Italian laboratories

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Organization of ring tests on diagnostic methods among Italian laboratories

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The occurrence of the olive quick decline syndrome (OQDS) in Puglia region represents one of the most serious plant health emergencies of recent years in which Italy and the entire European Union (EU) have had to deal with. Particularly crucial was the recovery of the quarantine pathogen *Xylella fastidiosa* in association with the disease, because it was never established in the EU territory before (Saponari *et al.*, 2013).

The need of official diagnostic protocols is a very critical issue when severe epidemics as the OQDS occur. A wide comparison among the available diagnostic methods is needed to obtain validation data of each method to be taken into consideration for the revision of the official diagnostic protocols (i.e. European Plant Protection Organization protocol). With this purpose a national test performance study (TPS) was coordinated by CREA-PAV, in the frame of an agreement with the Italian Ministry of Agriculture, by involving sixteen Italian laboratories that include several Plant Protection Services (PPS), SELGE, University of Milano, University of Catania, Centro di Ricerca, Sperimentazione e Formazione in Agricoltura (CRSFA), International Center for Advanced Mediterranean Agronomic Studies (CIHEAM of Bari). A working group was constituted, to define this interlaboratory comparison that included the following Institutions: CREA-PAV, (CNR-IPSP), PPS Lombardy, PPS Tuscany, PPS Liguria, PPS Veneto, PPS Emilia-Romagna, PPS Trentino Alto Adige, UNIMI.

The activity was organized in two steps: a PRE-TEST and the final TPS. The objectives of the PRE-TEST were the establishment of the analytical sensitivity of each method to select the bacterial contamination of samples and the methods to be assessed for the final TPS; moreover the repeatability, the analytical specificity, the relative accuracy were calculated. All performance criteria were elaborated following PM 7/76 (3) and PM7/98 (2) EPPO standards (EPPO 2014a, EPPO 2014b). The final TPS involved 16 laboratories to detect the reproducibility of the selected methods. For the development of PRE-TEST, three laboratories (CREA-PAV, CNR-IPSP, PPS Lombardy) received 16 samples consisting of two series of olive extract spiked with a devitalized *Xylella fastidiosa* CoDiRO strain suspensions ten-fold diluted from 10^7 cfu/ml to 10 cfu/ml. These samples, prepared by SELGE, consisted in: i) crude extracts to be used for direct analyses of ELISA (Agritest s.r.l.; Loewe s.r.l.) and LAMP (Enbiotech s.r.l.) and ii) olive extract to be extracted by C-TAB based method (Loconsole *et al*., 2014) to obtain total DNA for PCR (Minsavage *et al*., 1994), real-time PCR (Francis *et al*., 2006; Harper *et al*., 2010; IpadLab-Hyris s.r.l.) and LAMP (IpadLab-Hyris s.r.l. and Enbiotech s.r.l.). The real time PCR of the *cox* gene was used as internal control (Li *et al.,* 2006) obtaining 100% for all performance criteria.

As expected, the analytical sensitivity was lower when the crude extracts were used as target if compared with the total DNA. In particular, the values ranged from about 10^5 cfu/ml for the two ELISA tests to 10^3-10^4 cfu/ml for the LAMP (Enbiotech s.r.l.).

By using total DNA samples, the higher values (10-10^2 cfu/ml) were obtained by the real-time PCR and by real time LAMP (Enbiotech s.r.l.) processed in the ic-gene dedicated instrument. LAMP (IpadLab-Hyris s.r.l.) gave 10^2-10^3 cfu/ml whereas conventional PCR (Minsavage *et al.,* 1994) had lower sensitivity (10^4 cfu/ml).

The relative accuracy that consisted in the closeness of agreement between a test result and the accepted reference value (or the expected response from reference material) confirmed the higher reliability of real time LAMP (73%) with respect the two ELISA tests (respectively 63% Agritest s.r.l., and 56% Loewe s.r.l.) by using crude extract samples. The use of DNA as target showed the highest performance (100%) of the real-time PCR Francis *et al.* (2006) and the real time LAMP Enbiotech (s.r.l.). The repeatability was detected as the level of agreement between 5 replicates of a sample under the same condition, and resulted in: 100% for ELISA (Loewe s.r.l.), real-time PCR (Harper *et al.,* 2010 and Francis *et al.,* 2006), real time LAMP (Enbiotech s.r.l.). Conventional PCR (Minsavage *et al.,* 1994) gave 80% repeatability with undiluted DNA extracts and improve to 100% with ten-fold dilution DNA (probably due to an inhibition of PCR reactions).

Finally, exclusivity was assessed by CREA-PAV testing 36 different bacterial strains of several species. Real-time of Francis *et al*. (2006) gave aspecific or abnormal peaks with respectively *X. arboricola* pv. *celebensis* (NCPPB 1832), *Brenneria populi* (NCPPB 4299T) and B*. quercina* (NCPPB 1852^T), *Pantoea agglomerans* (ISF 438), *Pseudomonas marginalis* (CREA-PAV 1229), *X. hortorum* pv. *pelargonii*. Real-time PCR of Harper *et al.* (2010) did not show false positive results. The activity of the final TPS for the evaluation of reproducibility is currently in progress.

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