E

联合国粮食及农业组织 | Food and Agriculture Organization of the United Nations | Organisation des Nations Unies pour l'alimentation et l'agriculture | Продовольственная и сельскохозяйственная организация Объединенных Наций | Organización de las Naciones Unidas para la Alimentación y la Agricultura | منظمة الأغذية والزراعة للأمم المتحدة

COMMISSION ON PHYTOSANITARY MEASURES

Thirteenth Session

Rome, 16-20 April 2018

CPM recommendations - The application of Next Generation Sequencing technologies for plant pest diagnostics in a phytosanitary context

Agenda item 8.6

Prepared by Australia, EPPO and New Zealand.

I. Background

1. In December 2017, the Bureau considered a paper prepared by the Standards Committee (SC) which reflected discussion by the IPPC Technical Panel on Diagnostic Protocols (TPDP) on opportunities and challenges in relation to the use of Next Generation Sequencing (NGS) technologies as a diagnostic tool for phytosanitary purposes. The Bureau was asked to agree that the background paper be presented to CPM-13 with a request that the CPM note the challenges associated with the use of NGS technologies and that further work is needed on NGS technologies before they can be considered as the sole method for pest detection and identification.

2. The Bureau agreed that since this was an emerging issue that would be of interest to contracting parties, a CPM Recommendation should be drafted to provide policy advice and guidance to CPM on the use of NGS technologies as a diagnostic tool for phytosanitary purposes.

II. What is NGS and how is it different to other testing methods?

3. Next Generation Sequencing (NGS) technologies allow the sequencing of the whole genome and can be used for all types of organisms. NGS technologies can be used for targeted detection of regulated pests and also allow the detection of unknown organisms (i.e. without a priori knowledge). Indeed, application of these technologies has recently resulted in the discovery of previously undetected microorganisms, in particular viruses where the use of the technology is more advanced than for other pathogens (examples provided in this document are for viruses and viroids). Researchers and

diagnosticians using NGS technologies will continue to identify and describe new taxa due to the large volume of as yet undiscovered organisms. These technologies therefore enable a new and comprehensive approach to the detection and characterization of pests in a biological sample.

4. Phytosanitary testing for viruses and viroids in plants and plant products moved around the globe currently rely on a combination of specific (molecular and serological) and generic (visual, electron microscopy and biological indicators or bioassays) approaches. Whilst these methods are currently the best available they have a number of inherent weaknesses. The specific tests usually require a prior knowledge of the targeted viral pathogens and each test needs to be developed and validated (including validation of the test for different pest/host combinations), placing resource constraints on national plant protection organisations (NPPO). The host range of many pathogens is not well defined and exotic viruses and viroids that infect new plant species may not be detected in new pest-host combinations.

5. Whilst bioassays have traditionally been used to detect unknown viruses, further molecular or serological testing is usually required to confirm the identity of the causal agent when disease symptoms are observed. Bioassays are heavily reliant on environmental conditions for symptom expression and often produce ambiguous results as false positives and false negatives. Plants spend extended periods of time in post entry quarantine stations, significantly adding to costs and delays for importers. A further drawback with bioassays is that strains may not be detected if they are asymptomatic on the indicator host. Due to the limitations with traditional diagnostic methods, new robust, reliable and cost-effective methods are required to rapidly and reliably screen plants and plant products for viruses and viroids.

6. Studies conducted so far have demonstrated NGS to be equivalent or better than biological indexing assays in detecting viruses and viroids of agronomic significance (Rott, et al, 2017; Rwahnih et al. 2015; Mackie et al, 2017; Barrero et al. 2017). Most importantly the studies are demonstrating NGS is able to produce results significantly quicker than the time required for bioassays.

III. Regulatory and scientific challenges

7. Research findings based on NGS technologies may have significant implications within a phytosanitary framework. For example, there is a risk that plant material may be restricted in movement due to the perceived presence of a microorganism (e.g. virus) that may not have the potential to be pathogenic to its host. Not all organisms associated with plants are pests; some may be mutualists providing benefit to the host plant or commensal agents. There is also the issue, as with other indirect methods, NGS technologies will detect non-viable organisms.

8. The correct interpretation of results is one of the major challenges in using NGS in the phytosanitary context. Very large and well curated databases of sequence data of known pests and micro-organisms are required as the reference for comparison with NGS generated sequence data. NPPOs will need to make decisions on the basis of data analysis and not on information about the biological significance and ability to infect. This distances the diagnostic outcome from any analysis of pathogenicity and poses questions in deciding whether the data represents a biological entity that is a quarantine pest. However this same challenge is presented with current molecular sequencing methods particularly for ‘new to science’ viruses, so this is not a new problem. Other challenges in using NGS for regulatory purposes are noted by Massart et al (2017) and Martin et al (2016).

9. To give confidence to NPPOs in adopting NGS technologies for pest diagnosis, harmonized approaches are required including development of operational guidelines to reliably and repeatedly perform NGS including quality controls and validation data to interpret NGS outputs (Boonham et al, 2014). Validation of the technology against existing methods, which also take into account the limits of current procedures is also needed.

IV. Global collaboration

10. There are a number of initiatives underway globally that are exploring the use of NGS technologies as a diagnostic tool for phytosanitary purposes (for example in Australasia, Europe and North America). These include discussions on associated policies that may be developed. Co-ordination of outcomes from these initiatives is required to progress the timely development of internationally harmonised standards to use NGS in a regulatory setting.

11. The CPM Recommendation (Attachment 1) on "The application of Next Generation Sequencing technologies as a diagnostic tool for phytosanitary purposes" will provide a focus for this activity and is presented to CPM for consideration.

V. Recommendations

12. The CPM is invited to:

(1) *Note* the challenges associated with the use of the Next Generation Sequence (NGS) technologies and that further work is needed on NGS technologies before they can be considered as the sole method for pest detection;

(2) *Adopt* the CPM Recommendation on "The application of Next Generation Sequencing technologies as a diagnostic tool for phytosanitary purposes" (Attachment 1);

(3) *Establish* an international task force including NGS subject matter experts, policy regulators and TPDP members to identify benefits and impediments or constraints of adopting this technology from a regulatory perspective.

References

Barrero RA, Napier KR, Cunnington J, Liefting L, Keenan S, Frampton RA, Szabo T, Bulman S, Hunter A, Ward L, Whattam, M and Bellgard, M (2017) An internet-based bioinformatics toolkit for plant biosecurity diagnosis and surveillance of viruses and viroids. BMC Bioinformatics, 18:26. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5225587/

Boonham N, Kreuze J, Winter S, van der Vlugt R, Bergervoet J, Tomlinson J, and Mumford R. (2014) Methods in virus diagnostics: from ELISA to next generation sequencing. Virus Res. 24 186:20-31. https://www.ncbi.nlm.nih.gov/pubmed/24361981

Mackie J., Liefting L., Barrero, R.A., Dinsdale, A., Napier, K.R., Blouin, A.G., Woodward, L., Khan S., Bellgard, M.I., Ward L. and Whattam, M. (2017) Comparative diagnosis of viral pathogens using side-by-side trials of existing Post Entry Quarantine and small RNA next generation sequencing methods. Abstract PBCRC Science Protecting Plant Health 2017 conference http://apps-2017.p.yrd.currinda.com/days/2017-09-26/abstract/4017

Martin, R.R, Constable, F. and Tzanetakis, I.E. (2016) Quarantine Regulations and the Impact of Modern Detection Methods. Ann. Rev. Phytopath. Vol. 54:189-205 http://www.annualreviews.org/doi/abs/10.1146/annurev-phyto-080615-100105

Massart S, Candresse T, Gil J, Lacomme C, Predajna L, Ravnikar M, Reynard JS, Rumbou A, Saldarelli P, Škorić D, Vainio EJ, Valkonen JP, Vanderschuren H, Varveri C, Wetzel T. (2017) A framework for the evaluation of biosecurity, commercial, regulatory and scientific impacts of plant viruses and viroids identified by NGS technologies. Front Microbiol. 2017 Jan 24;8:45 https://www.ncbi.nlm.nih.gov/pubmed/28174561

Rott, M., Xiang, Y., Boyes, I., Belton, M., Saeed, H., Kesanakurti, P., Hayes, S., Lawrence, T., Birch, C., Bhagwat, B. and Rast, H. (2017) Application of Next Generation Sequencing for Diagnostic Testing of Tree Fruit Viruses and Viroids. Plant Disease 101:1489-1499

https://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-03-17-0306-RE

Rwahnih M. A., Daubert, S., Golino, D., Islas, C. and Rowhani, A. (2015) Comparison of Next-Generation Sequencing Versus Biological Indexing for the Optimal Detection of Viral Pathogens in Grapevine. Phytopathology 105:6:758-763 https://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO-06-14-0165-R

Attachment 1

RECOMMENDATION TEXT

Recommendation on:

The application of next generation sequencing technologies as a diagnostic tool for phytosanitary purposes

BACKGROUND

The Commission on Phytosanitary Measures (CPM) recognizes that accurate and timely pest diagnosis underpins export certification, import inspections and the application of appropriate phytosanitary measures[1]. It is widely accepted that the ability to detect and identify a plant pest varies with the quality and specificity of the detection tools.

Next Generation Sequencing (NGS) technologies, also known as high throughput sequencing, have provided a powerful alternative for detection and identification of organisms with a *priori* knowledge. However, these diagnostic outcomes may not be associated with evidence of living pests or damage to the plant or plant products by these organisms. As such, the use of highly sensitive technologies, such as NGS, for the detection and identification of plant pests must be introduced cautiously and with due consideration of the risks and consequences of applying diagnostic outcomes to regulate phytosanitary risks.

ADDRESSED TO

Contracting parties and regional plant protection organizations.

RECOMMENDATIONS

The Commission notes findings based on NGS technologies of an unknown microorganism need to be further investigated to demonstrate the potential of that microorganism to be a plant pest and that would qualify as a regulated pest. The Commission notes that there are existing challenges and further work is needed on NGS technologies before they can be considered as the sole method for pest detection.

To improve the capacity and capability of contracting parties to adopt NGS technologies, the Commission *encourages* contracting parties and regional plant protection organizations to:

(a) *Consider and agree* on establishing an international task force including NGS subject matter experts, policy regulators and TPDP members to identify benefits and impediments or constraints of adopting this technology from a regulatory perspective;

(b) *Consider and agree* on establishing a specific timeframe, set out by the task force, setting out clear milestones with the objective of international adoption of NGS for routine phytosanitary screening of viruses and viroids;

(c) *Actively engage and support* international efforts in developing and finalising standardised operational guidelines for NGS including proper interpretation of results and agreed quality control measures to ensure NGS data outputs are robust and accurate;

(d) *Support* international efforts in obtaining more scientific evidence on reliability and accuracy of NGS by conducting trials comparing NGS against existing diagnostic platforms;

---

[1] See also CPM recommendation R-07: The importance of pest diagnosis (https://www.ippc.int/en/publications/84234/)

(e) *Share* knowledge and expertise with other countries where possible and support the development of NGS training programs including delivery of best laboratory practice courses online and co-ordinating international proficiency testing to independently assess laboratory capability; and

(f) *Share* agreed international NGS protocols and training material on the IPPC phytosanitary resources page once finalised.

RECOMMENDATION(S) SUPERSEDED BY THE ABOVE

None.