22-Improvement of three nucleic acid isolation protocols for an overall diagnosis of viruses on six vegetative propagated plants

Julian C.[1], Bernardo P.[1], Fernandez E.[1], Galzi S.[1], Grisoni M.[2], da Silva Mendoça DM.[3], Pavis C.[4], Roumagnac P.[1], Filloux D.[1]

[1] INRA-CIRAD-SupAgro. UMR BGPI TA A54/K Campus International de Baillarguet F-34398 Montpellier Cedex 5.
[2] CIRAD, UMR PVBMT, Pôle de Protection des Plantes, Saint Pierre, La Réunion, France
[3] Biotechnology Centre of Azores / University of Azores, Rua Capitão João d'Ávlia – Pico da Urze. 9700-042 Angra do Heroísmo. Azores. Portugal
[4] INRA, UR1321 ASTRO AgroSystèmes TROpicaux, Domaine Duclos, F-97170 Petit-Bourg (Guadeloupe), France

charlotte.julian@supagro.inra.fr

Biological Resources Center (BRCs) must be able to guarantee the sanitary status of the resources they distribute, in order to prevent the spread or emergence of diseases. However, BRCs' vegetatively propagated crops do not benefit from the partial sanitation occurring through a seed cycle. This is particularly a problem for viral diseases, which have an overall high prevalence in vegetatively propagated crops. Variously effective sanitation methods exist for recovering virus-free plants but their successful implementation depends on on the availability of sensitive, polyvalent and reliable diagnosis tests for all relevant virus species.
The main objective of the SafePGR project is to improve the knowledge of the diversity of viruses infecting the vegetatively propagated crops addressed by the partners' BRCs (Universidad do Açores, Universidad da Madeira, INRA-CIRAD Guadeloupe and CIRAD La Réunion). Among the various issues addressed in achieving the goals of the SafePGR project, we need to develop new tools for an overall diagnosis of viruses. Thus, recent metagenomics methods associated with high-throughput sequencing will be tested. For this purpose, we started to develop and adapt three different nucleic acids extractions on six plants species: banana, garlic, sugarcane, sweet potato, vanilla and yam. First, we succeeded to extract small RNAs using Trizol or phenol:chloroform methods on these six species. Then, we have developed a protocol to semi-purify viral particles. The third protocol consisted in an enrichment of double-stranded RNAs. The quality and quantity of extracted nucleic acid varied among plant species. Overall, the extracted RNAs from garlic, sugarcane, sweet potato and vanilla were fulfilling criteria of quality and quantity for being used for metagenomic approaches whereas the ones from banana and yam were not adequate. These preliminary results tend to indicate that it would be probably difficult to develop a universal nucleic acid isolation method that could be routinely used by our partners' BRCs.