Implementation of new techniques for diagnostics in quarantine IAC

https://doi.org/10.56238/interdiinovationscrese-033

Barbara Negri
Campinas Agronomic Institute (IAC)/Phytosanitary Research and Development Center. Campinas, sp.

Martha Maria Passador
Campinas Agronomic Institute (IAC)/Phytosanitary Research and Development Center. Campinas, sp.

Roberta Pierry Uzzo
Campinas Agronomic Institute (IAC)/Phytosanitary Research and Development Center. Campinas, sp.

Christina Dudienas
Campinas Agronomic Institute (IAC)/Phytosanitary Research and Development Center. Campinas, sp.

Julieta Andrea Silva de Almeida
Campinas Agronomic Institute (IAC)/Coffee Research and Development Center "Alcides Carvalho".

ABSTRACT
The IAC Quarantine Complex is responsible for carrying out the quarantine of plant materials that will be introduced in Brazil by private companies or public institutions, with the objective of scientific research. The materials are analyzed for pathogenic agents, as well as sown in a greenhouse for growth analysis and to verify possible pathogen symptoms. Some viruses, bacteria, and fungi, present in imported materials, require increasingly improved diagnostic and traceability techniques. As of November 2012, implementations began to promote the development of this work within a modern structure, based on international quarantine models. Including adaptations of greenhouses, cold chambers, organization, and assembly of new laboratories for the analysis of materials, in this way, new methodologies were studied and applied, such as techniques involving molecular biology (PCR). To complement the efficiency of the analyzes carried out in plant quarantine, more specific and precise tests were developed. For better results and greater security, the analyzes are carried out within IAC Quarantine complex.

Keywords: Plant Quarantine, Molecular biology, Scientific research.

1 INTRODUCTION

Plant quarantine is a biosecurity measure and can be defined as all activities designed to prevent the introduction and spread of pests of economic importance that are not yet present in free areas, or that are present but not widely distributed and officially controlled (FAO, 2007; Marques et al., 2016). Within this context, it is an activity that aims to prevent the spread of exotic pests, through the control of plant material coming from suspected countries or regions, whose health status poses doubts (Marinho et al., 2004).

The IAC Quarantine is responsible for quarantines of genetic materials with the aim of detecting and containing the introduction and dissemination in the country of pests and diseases of economically important plants, or plant products that are not yet present in an area, or that are present but not occur widely and are under official control. After inspections by the Ministry of Agriculture,

Livestock and Supply (MAPA), in accordance with Ordinance No. 56 of May 11, 1998, DO No. 91 of May 15, 1998, it became accredited to carry out plant quarantine (Veiga et al., 2005).

The analyzes of the samples received (inspection, quarantine and release of material free from exotic pathogens) are supported by an Internal Quarantine Committee, which is made up of researchers from the Agronomic Institute (IAC), the Biological Institute (IB) and external professionals. experts in related areas (Dudienas et al., 2020). The materials are analyzed for pathogens, as well as sown in a greenhouse to analyze growth and check for possible symptoms of diseases or signs of phytopathogens (Ministry of Agriculture, Livestock and Supply, 2022). Pathogens such as viruses, bacteria and some fungi present in imported materials require increasingly improved diagnostic and traceability techniques.

The materials received consist of different varieties of plants for the purpose of study, research, and improvements in production. They can be in the form of seeds and cuttings, seedlings in substrate, and plants grown in vitro. In view of the above, the present study aimed to disseminate new diagnostic techniques in plant quarantine developed in the new facilities, and the results obtained so far.

2 MATERIALS AND METHODS

The materials received at the IAC Quarantine are analyzed through seed health tests, evaluation of the presence of insects, mites, weeds, nematodes, fungi, bacteria and viruses, in samples taken from the source material. Parallel to the planting stage, carried out in greenhouse conditions adapted to the needs of a quarantine, laboratory tests are carried out such as seed pathology, PCR and identification of bacteria and fungi (Doyle; Doyle, 1987; Sigma Aldrich, 2019; Passador et al., 2018).

As a first step towards receiving and analyzing the materials that will be quarantined, the company or research institution that is importing said materials must send an email to the quarantined person requesting a letter of acceptance. This letter contains information related to origin and origin, quantity of plant material, name of the crop, expected date of arrival, suitable temperature for storage, and also whether they are genetically modified organisms (GMO).

The conditions for receiving the plant material are checked, such as available space and what analyzes will be required. A response is then sent to the company with the acceptance letter issued. It is important to highlight that the quarantine procedures are in accordance with the rules of the Ministry of Agriculture, Livestock and Supply (MAPA), which issues import permission to the requesting company or research institution.

All material transit is monitored by the applicant. The country of origin sends the material and upon arrival at the airport, MAPA carries out an inspection, following which the plant material is transported in its entirety to the quarantine facility that issued the letter of acceptance.

The material is received and checked within the quarantine facilities. A list with identification and quantity accompanies the load, this is used to check the material, the physiological conditions of the seeds, plants, bottles and cultivation media are also observed. If there is any non-compliance, this is reported to MAPA, which will indicate the procedures for each situation.

After verification of receipt, specialists (entomologist, matologist and nematologist) are called to come to the quarantine to analyze the material. If no pathogens have been observed, sampling is carried out, where up to 10% of the quantity of bottles and/or plant material is separated for analysis of phytopathogens (viruses, fungi and bacteria).

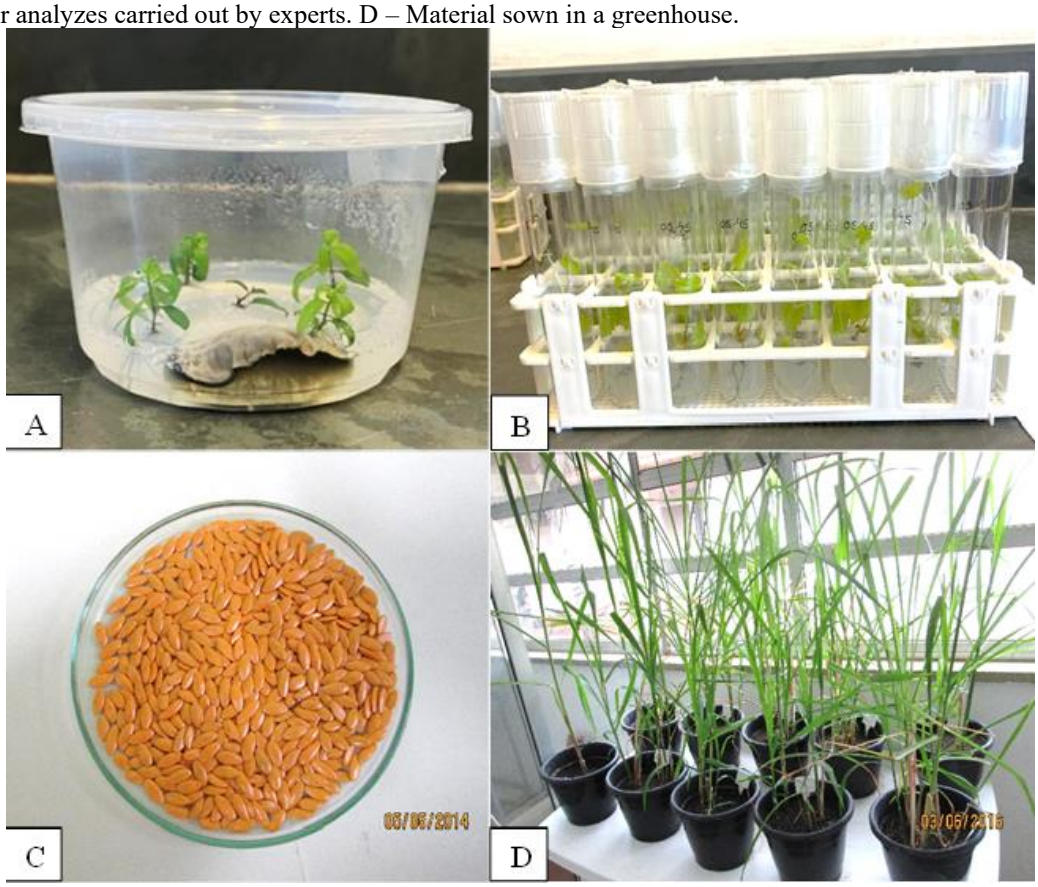
For in vitro quarantine, it can contain from 10 to numerous plastic tubes or pots, containing one or more seedlings, which are kept in air-conditioned rooms with a photoperiod according to the needs of the culture, and these conditions are checked daily. The tissue culture specialist performs a visual analysis with the aid of a magnifying glass to check the quality of the culture medium and the presence of contaminants, as well as the condition of the plants and signs of the presence of pathogens. In some cases, such procedures may require more time, and some companies request the transfer of seedlings to a new growing medium during the quarantine period, as the original growing medium may dry out or the plants may grow excessively. Some quarantines arrive with contaminated vials, these are eliminated through autoclaving (40 minutes at 121ºC). Some vials (not exceeding 10% of the quantity received) are sent for laboratory analysis to check for the possibility of infection by fungi, bacteria and/or viruses (Figure 1A and 1B).

For other plant materials such as seeds (Figure 1 C), seedlings, cuttings, among others, a visual analysis is carried out by the entomologist and/or matologist and nematologist. After this analysis, a sample of the material or the entire load (seedling, billet, stake) is sent to the greenhouse for planting (Figure 1D). During this period, it is checked whether there is a need for any phytosanitary treatment after the aforementioned analyses. For activities in a greenhouse, protocols related to the use of coats and boots are followed, and for greater safety of quarantined materials, the rooms are individualized.

Phytopathology and virology specialists are those who monitor plant growth and carry out final analyzes to determine whether or not plant material is released.

The tests are carried out mainly looking for pathogens that are absent in Brazil, according to the list of Absent Quarantine Pests (Ministry of Agriculture, Livestock and Supply, 2022) and are carried out by specialists in the respective areas. The presence of viruses is detected by PCR and, in situations where amplifications occur that result in amplicons, the material will be sent for sequencing, in order to identify the amplified fragment.

Figure 1. A and B - In vitro materials received for analysis, with and without contamination, respectively C - Seeds separated for analyzes carried out by experts. D – Material sown in a greenhouse.



If a pathogen or pest is found in Brazil, the plant materials are subjected to phytosanitary treatments, recommended by the pest specialist. After analyzing these reports, MAPA will send a letter authorizing the release of the material, so that it can be removed by the company or research institution.

To carry out this study, the information obtained about the materials received by the IAC Quarantine, was from January 2013 to November 2018, coming from a database, which consists of acceptance letters, material registration forms and laboratory reports. It only considered crops, not the amount of access.

In the plant quarantine process, to check possible symptoms of pathogens as well as their presence, the materials were analyzed visually and using molecular biology techniques (PCR).

To complement the efficiency of these analyses, it is necessary for more specific and precise tests to be carried out when symptoms and signs of a pathogen are present, as well as in their absence. Within this context, diagnoses involving molecular biology techniques using PCR will provide faster results for the quarantine service.

These activities will be carried out in the Molecular Biology laboratory of the IAC Quarantine, recently implemented in accordance with the requirements of MAPA (IN 28, Ministry of Agriculture, Livestock and Supply, 2020), and from pre-existing facilities at the Agronomic Institute. In this way, the work carried out in this sector will be carried out simultaneously with the analyzes carried out by

members who make up the quarantine committee of the Agronomic Institute. These members are experts in entomology, phytopathology, virology, nematology, seed pathology, weeds and tissue culture.

This class of diagnosis will allow results and reports to be released to companies more quickly. Preference will be given to nucleic acid extraction methods that can be used in the daily quarantine routine, even for a large amount of plant material to be analyzed. To this end, protocols available in the literature will be tested and used, which provide greater detection performance and offer reliable results. After extracting the nucleic acids, the PCR technique and the sequencing technique will contribute to obtaining the desired diagnoses. Extractions will take place from plant material, however for some fungi and bacteria it is necessary to cultivate them in specific culture media for each microorganism. Depending on each situation, there may be a need to develop and/or modify protocols already available and published, so as to provide an increasingly efficient service, and which could be a procedure to be adopted by other quarantine facilities and/or accredited laboratories.

When an absent quarantine pest (PQA) is detected, a notification is sent to MAPA, which can determine whether or not to destroy the material.

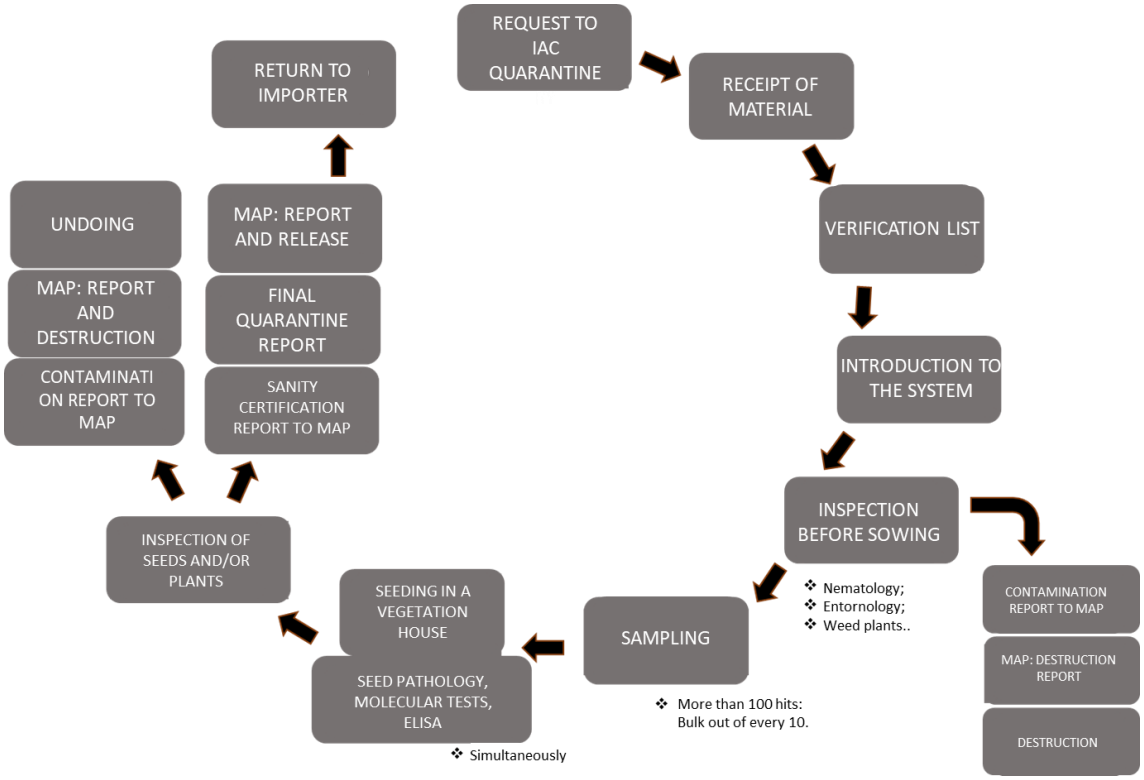
Upon completion of all analyses, all expert reports and the final report are sent to MAPA. For plant material, such as: seedlings, cuttings, in vitro material, and genetically modified materials, a list is also included. In most quarantines, a molecular biology analysis report is prepared.

3 RESULTS AND DISCUSSION

In addition to boosting diagnostic services, this is a specialized structure organized according to procedures or protocols that include phytosanitary safety, guaranteeing identity and traceability.

The work carried out both in daily routine and in research, allows the preparation of works that can be presented at congresses and scientific meetings, as well as the publication of manuscripts containing results of activities carried out in the laboratory, such as quantity and types of materials received, microorganisms most common, among others (Figure 2). Likewise, relevant information may be grouped in a publicly accessible catalog, available on the IAC website, in a way that contributes to studies carried out in companies and educational and research institutions.

Figure 2. Descriptive flowchart of the Plant Quarantine process, from receipt of all plant material to its release or destruction.



The structures of the new laboratories were designed in accordance with MAPA specifications. After completion of the structural installations, the necessary items to carry out the activities were purchased (Figure 3). In addition, maintenance and inspection of the laboratories was also carried out to ensure their proper functioning, and ensuring their organization and cleanliness.

Sampling and analysis are carried out within new laboratories (Figure 4A and 4B), in sectors organized according to use and to make better use of available spaces. These sectors range from receiving materials, opening boxes and packages, sorting and distribution to analysis laboratories and specialist members.

Sampling and analysis are carried out within new laboratories (Figure 4A and 4B), in sectors organized according to use and to make better use of available spaces. These sectors range from receiving materials, opening boxes and packages, sorting and distribution to analysis laboratories and specialist members.

Figure 3. Molecular Biology Laboratory, including electrophoresis room. A - Bench with thermocycler and pH meter. B - Workbench with equipment and sinks. C - Workbench where reactions are prepared, with equipment and micropipettes. D - Electrophoresis room with tank and photodocumentator. E - Ultrapure water room with respective equipment. F and G - Autoclave and ultrafreezer (-80ºC).

Figure 4. A - Laboratory for opening boxes and sorting seeds. B – Access door to laboratories with wind curtain.

The implementation of new structures for laboratories and new techniques for diagnosing phytopathogens allowed results to be obtained, contributing to the scientific production of the IAC Quarantine Center, with works presented and scientific meetings and a book chapter (Dudienas et al., 2020; Passador et al., 2018; Passador et al., 2019; Passador et al., 2021; Passador et al. 2022a, 2022b; 2022c; 2022d).

REFERENCES

DOYLE, J.J.; DOYLE, J.L. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochemical Bulletin, v.19, p. 11-15, 1987

DUDIENAS, C.; UZZO, R.P.; PASSADOR, M.M. Quarentenário IAC. O Agronômico, v. 72, p. 96-98, 2020

FAO. Framework for pest risk analysis. International Standards for Phytosanitary Measures. N. 2, Food Agric. Organ, Rome. 2007

MARINHO, V. L.; MENDES, M.A.S; BATISTA, M.F.; FONSECA, J. N.L. Quarentena Vegetal: Histórico, conceitos e definições. In: Fungos quarentenários para o Brasil. MENDES, M.A.S.; FELIX, A.A.A.; SANTOS, M.F.; HERNÁNDEZ GUTIÉRREZ, A. (Eds.). Brasília: Embrapa Recursos Genéticos e Biotecnologia, p. 13-18, 2004

MARQUES, A. S. DOS A.; LOPES-DA-SILVA, M.; GONZAGA, V.; FERNANDES, F. R.; BENITO, N. P.; VEIGA, R. F. DE A. Fundamentos biológicos, ferramentas operacionais e inovação em quarentena vegetal. Pesquisa Agropecuária Brasileira, v. 51, n. 5, 483-493, 2016

Ministério da Agricultura, Pecuária e Abastecimento. Mapa. Portaria nº 617, de 11 de julho de 2022. Diário Oficial, 2020

Ministério da Agricultura, Pecuária e Abastecimento. Mapa. Instrução Normativa nº 28, de 20 de abril de 2020. Atualiza lista de pragas quarentenárias ausentes (PQA) para o Brasil. Diário Oficial, 2022

PASSADOR, M. M.; YUKI, V. A.; MARUBAYASHI, J. M.; ITO, M. F.; UZZO, R. P.; DUDIENAS, C. Coleta e preservação de controles positivos para diagnose de fitopatógenos no Quarentenário/IAC. In: 41º Congresso Paulista de Fitopatologia, 2018, Marília

PASSADOR, M. M.; MARUBAYASHI, J. M.; UZZO, R. P.; DUDIENAS, C; YUKI, V. A. Padronização de protocolos de extração de ácidos nucleicos na rotina de diagnósticos em quarentena vegetal. In: 51º Congresso Brasileiro de Fitopatologia, 2019, Recife. Tropical Plant Pathology. Brasilia: Sociedade Brasileira de Fitopatologia (SBF), 2019

PASSADOR, M. M.; UZZO, R. P.; YUKI, V. A. DUDIENAS, C. Preservação de controles positivos do gênero *Potyvirus* para análises em quarentena vegetal. In: IX Congresso Virtual de Agronomia, 2021, Curitiba. IX Congresso Virtual de Agronomia, 2021

PASSADOR, M. M.; UZZO, R. P.; MARUBAYASHI, J. M.; YUKI, V. A.; DUDIENAS, C. Adaptação de protocolo de RT-PCR para análises de vírus do gênero *Potexvirus*. In: X Congresso Virtual de Agronomia, 2022, Curitiba. X Congresso Virtual de Agronomia, 2022a.

PASSADOR, M. M.; UZZO, R. P.; MARUBAYASHI, J. M.; YUKI, V. A.; DUDIENAS, C. Adaptação de protocolo de RT-PCR para análises de vírus do gênero *Potexvirus*. In: Everton Nogueira Silva. (Org.). Multiplicidade das Ciências Agrárias. 1ed.Fortaleza: Editora In Vivo, 2022b, v. 3, p. 58-65.

PASSADOR, M. M.; UZZO, R. P.; NEGRI, B.; YUKI, V. A.; DUDIENAS, C. Aplicações da biologia molecular para análises de sementes no Quarentenário IAC no período de janeiro de 2018 a maio de 2022. In: XXI Congresso Brasileiro de Sementes, 2022, Curitiba. Anais do XXI Congresso Brasileiro de Sementes. Londrina: ABRATES, 2022c. v. 29. p. 128-128.

PASSADOR, M. M.; UZZO, R. P.; MARUBAYASHI, J. M.; YUKI, V. A.; DUDIENAS, C. Preservação de controles positivos do gênero *Tobamovirus* para análises em quarentena vegetal. In: VI Congresso Brasileiro de Fitossanidade, 2022, Goiânia. Anais do VI Congresso Brasileiro de Fitossanidade, 2022d

SIGMA ALDRICH (n.d.). Tri Reagent. Technical Bulletin. Retrieved January 16, 2019, from https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/t9424bul.pdf

VEIGA, R. F. A.; BARBOSA, W.; TOMBOLATO, A. F. C.; COSTA, A. A.; BENATTI JUNIOR, R. Quarentenário de Plantas no Instituto Agronômico. O Agronômico, v. 56, p. 24-26, 2005