Submissions for Diagnostic Protocols

1. General information

Submission number	2021-017
Title of Proposal	DRAFT ANNEX to ISPM 27 - Tomato brown rugose fruit virus
Submitted by	IPPC Contracting Party China
Submission supported by	China

2. Contact information

Name	Xiuling Yang
Position and organization	Institute of Plant Protection, Chinese Academy of Agriculture Sciences P.R. China
Mailing address	No.2 Yuanmingyuanxi Road Haidian Beijing China
Phone	0086-10-62816620
Email	ippc@agri.gov.cn

3. Summary of proposal

Summary of justification for the proposal	Tomato brown rugose fruit virus (ToBRFV) is a new species of the genus Tobamovirus, which was first observed in 2014 and 2015 on tomatoes in Israel and Jordan. It can be easily transmitted by contact, propagation materials, seeds, and bumblebees. Since its first discovery, ToBRFV has spread rapidly and its incidence has been reported globally in only a few years. Infection of tomato plants by ToBRFV reduces the vigour of the plant, causes yield losses and virus symptoms make the fruits unmarketable. The rapid international spread of ToBRFV combined with its ability to break Tm-22-mediated resistance genes is causing alarm within the see production industry and tomato growers worldwide. As a result, ToBRFV was added to the EPPO Alert List and is listed as a quarantine species by many countries. As ToBRFV is easily spread and difficult to control and eradicate, prevention and early diagnosis of ToBRFV is critical to safeguard tomato production. Although different approaches have been developed for the molecular identification of ToBRFV, the reliability, reproducibility, and performance of different methods are confusing. To be effective, a harmonization of the diagnostic techniques for ToBRFV must be developed and internationally accepted. The IPPC, through an Annex to ISPM27, provides the only viable platform to achieve international acceptance of a globally harmonised diagnostic protocol to monitor the distribution of ToBRFV and to determine strategies of virus management.
Proposed priority	1 (high)
Comments	ToBRFV is a new member of the genus Tobamovirus, and has the potential to affect the production and marketability of tomatoes and peppers. Due to its strong infectivity, outbreaks of ToBRFV have occurred in many countries such as Mexico, the United States of America, Germany, Italy, Palestine, Turkey, United Kingdom, Greece, China, Spain, Holland, France, Czech Republic, and Cyprus in only a few years. As plant viruses are currently not curable, growing resistant plant varieties has been demonstrated to be the most effective strategy to control plant virus infection. However, in tomato, the long lasting resistance genes, Tm-1, Tm-2, Tm-22, which have been proved to provide complete resistance against several tobamoviruses such as tobacco mosaic virus and tomato mosaic virus, cannot confer resistance to ToBRFV. No commercial varieties are currently known to be fully resistant to ToBRFV. Furthermore, ToBRFV can be easily transmitted by seed, mechanical contact, and bumblebees. Therefore, ToBRFV could potentially establish in the regions wherever tomatoes are grown and is likely to cause significant economic impact in many countries via different pathways. According to the rapid risk assessment (RPA), ToBRFV was added to

	the EPPO A2 List of pests recommended for regulation as quarantine pests in 2020. The Panel on Phytosanitary Measures suggested ToBRFV as the highest priority for an EPPO RPA in 2019. RPAs for ToBRFV have also been performed in France, Germany, Italy, the United Kingdom, Mexico, and China. Measures are encouraged to be implemented by both the exporting and importing countries. Considering the high risk of ToBRFV, ToBRFV is herein proposed as the highest priority, and an international diagnosis protocol for ToBRFV should be developed. The proposed diagnosis protocol would provide guidance to an early detection and control of ToBRFV, which will benefit seed industry and tomato growers at a global level.

4. Literature review

Literature review	Tomato brown rugose fruit virus (ToBRFV) is a new member of the genus Tobamovirus. It was first discovered in Israel and Jordan in 2014 and 2015, and first characterized in 2016. Since its first discovery, the incidence of ToBRFV has been reported to occur in many countries such as Mexico, the United States of America, Germany, Italy, Palestine, Turkey, United Kingdom, Greece, China, Spain, Holland, France, Czech Republic, and Cyprus in only a few years. As with other tobamoviruses, ToBRFV has a positive single-stranded RNA genome that is encapsidated into rod-shaped particles of about 300 nm in length and 18 nm in diameter. The genome of ToBRFV is about 6,390 nucleotides, containing four predicted open reading frames (ORFs). Two ORFs (ORF1 and ORF2) code for replication-related proteins of 126 and 183 kDa, ORFs encode a 30-kDa movement protein, and ORF4 encode the coat protein of approximately 17.5 kDa. ToBRFV can be easily transmitted to healthy plants by mechanical contact via common cultural practices or by the action of bumblebees. It can also contaminate the seed coat and can be spread over long distances by the seed. Similar to other tobamoviruses, the viral particles of ToBRFV are very stable and can exist in soil where previously infected plants were grown and can survive on surfaces such as plant debris or agricultural materials for a long time. When infected by ToBRFV, tomato plants display mosaic and deformation symptoms on leaves, and yellow spots or rough dead patches on tomato fruits, leading to severe yield losses and unmarketability of fruits. ToBRFV was reported to infect tomato and pepper plants in nature, but can infect and induce symptoms in Nicotiana benthamiana, N. tabacum cv. Samsun nn plants. ToBRFV is an intracellular parasite that relies on the host machinery for its life cycle. Unlike some plant-infecting bacteria and fungi, ToBRFV and other plant viruses are not curable and control of ToBRFV depend largely on prophylactic measures. Growing resistant plant varieties has been demonstrated to be the most effective strategy to control plant virus infection. However, in tomato, the long lasting resistance genes, Tm-1, Tm-2, Tm-22, which have been proved to provide complete resistance against several tobamoviruses such as tobacco mosaic virus and tomato mosaic virus, cannot confer resistance to ToBRFV. No commercial varieties are currently known to be fully resistant to ToBRFV. Therefore, preventing viruses from entering plants by quarantine, certification, and removal of infected plants are crucial in combating epidemics of ToBRFV in tomatoes. A precise and definitive diagnosis is of utmost importance as a first step to control ToBRFV in crops. This is especially important in the cases of diseases that have only been recently introduced and in ToBRFV-free regions or contries. Currently, the diagnosis and detection of ToBRFV have been performed through transmission electron microscopy (TEM), deep sequencing, sequence-specific reverse-transcription polymerase chain reaction (RT-PCR) primers, real-time RT-PCR, loop-mediated isothermal amplification, CRISPR/Cas12a, and serological methods such as ELISA. However, the reliability, reproducibility, and performance of different methods are confusing. For example, as all tobamoviruses have rod-shaped viral particles, TEM-based approach cannot be used to distinguish ToBRFV from closely related tobamoviruses such as tobacco mosaic virus and tomato mottle virus. Due to the high conservation of the tobamovirus coat protein, antibody cross reactivity has been often observed between tobamovirus species. Due to the globalization of the international markets of pepper and tomato, a standardized and precise diagnostic method is emerging required to cope with this challenge and to safeguard tomato production and global tomato and pepper seed trade.

5. Criteria for prioritization of Diagnostic Protocols

Criteria	Information provided by Submitter
1. Need for international harmonization of the diagnostic techniques for the pest (e.g. due to difficulties in diagnosis or disputes on methodology)	ToBRFV is an intracellular parasite that relies heavily on host machinery. Unlike bacteria or fungi that can be treated with antibacterial or antifungal agents, respectively, curing plants that have been infected by ToBRFV is not feasible. A fast and accurate diagnosis of ToBRFV is of utmost importance as a first step to control ToBRFV in crops. This is especially important in the cases of diseases that have only been recently introduced. Currently, the diagnosis and detection of ToBRFV have been performed through transmission electron microscopy (TEM), deep sequencing, sequence-specific reverse-transcription polymerase chain reaction (RT-PCR) primers, real-time RT-PCR, loop-mediated isothermal amplification, CRISPR/Cas12a, and serological methods such as ELISA. However, the reliability, reproducibility, and performance of different methods are confusing. For example, as all tobamoviruses have rod-shaped viral particles, TEM-based approach cannot be used to distinguish ToBRFV from closely related tobamoviruses such as tobacco mosaic virus and tomato mottle virus. Due to the high conservation of the tobamovirus coat protein, antibody cross reactivity has been often observed between tobamovirus species. To cope with this challenge, establishment of a standardized diagnostic method is necessary, due to the globalization of the international markets of pepper and tomato.
2. The relevance of the diagnosis to the protection of plants including measures to limit the impact of the pest.	As strict intracellular pathogens, ToBRFV cannot be controlled chemically and prophylactic measures are vital to limit the occurrence and spread of ToBRFV. Use of plant varieties carrying genetic resistances constitutes the most effective, economical, and ecological measure to control ToBRFV. However, ToBRFV can break the long lasting resistance genes-mediated resistance in tomato, and no commercial complete resistant tomato varieties are currently available. Therefore, preventing viruses from entering plants by quarantine, certification, and removal of infected plants are crucial in combating epidemics of ToBRFV in tomatoes. Symptom observation has been the most common and simplest way to monitor ToBRFV infection in plants in the field. However, alike symptoms can be caused by ToBRFV and different plant viruses such as pepino mosaic virus and tobacco mosaic virus. Variations in symptoms sometimes due to variation in environmental factors, the cultivar, and the virus strain may lead to an improper diagnosis. Therefore, a precise and definitive diagnostic method is central to guiding choice of treatment measures and monitoring the resistance of different varieties to ToBRFV. A standard diagnosis protocol is very important for protection of tomato and pepper plants from ToBRFV infection and enhancement of the ecological, economic, and social importance of tomato and pepper varieties.
3. Importance of the plants protected on the global level (e.g. relevant to many countries or of major importance to a few countries).	Tomato is one of the world's most cultivated and extensively consumed vegetable crops, with an annual production of about 180 million tons in 2019. In terms of economical value, tomato constitutes about 70% of the value of fresh vegetables worldwide. Despite the increased annual production of tomatoes over the past five years, the yield and fruit quality of tomato plants are largely affected by an array of pests and diseases during their growing season. ToBRFV is one of such pests that are posing a serious threat to global tomato production. Therefore, protecting tomato plants from ToBRFV infection will benefits tomato growers and seed industry in many countries.
4. Volume / importance of trade of the commodity that is subjected to the diagnostic procedures (e.g. relevant to many countries or of major importance to a few countries).	The value of exported tomatoes increased by an average 12.9% for all shipping countries since 2016. In 2020, the global sales for tomatoes exported by all countries were estimated to be US$9.8 billion. Mexico, Netherlands, Spain, Morocco, and Canada constitute the top five countries that exported the highest dollar value worth of tomatoes during 2020. Currently, ToBRFV has been listed as a quarantine pest by many countries. Therefore, diagnosis of ToBRFV is important in both import and export of tomatoes at a global level and facilitating safe trade globally.
5. Other criteria for topics as determined by CPM that are relevant to determining priorities	-

Criteria	Information provided by Submitter
6. Th balance between pests of importance in different climatic zones (temperate, tropics etc) and commodity classes.	ToBRFV can be easily transmitted by seed, mechanical contact, and bumblebees. When introduced abroad, ToBRFV could potentially establish in the regions wherever tomatoes are grown and is likely to cause significant economic impact in many countries via different pathways.
7. Number of labs undertaking the diagnosis.	Diagnosis and management of ToBRFV is a matter of viral importance and concern to farmers, horticulturists, manufacturers as well as plant virologists. Labs from different institutes and universities, seed companies, and quarantine departments are undertaking the diagnosis of ToBRFV.
8. Feasibility of production of a protocol, including availability of knowledge and expertise.	The appropriate diagnosis protocol is important for controlling ToBRFV and facilitating safe trade globally. Although ToBRFV is relatively new to science, some published scientific information is available. These publications are mainly focused on development of different detection approaches and molecular identification of ToBRFV in different countries. Currently, several approaches such as deep sequencing, sequence-specific reverse-transcription polymerase chain reaction (RT-PCR) primers, real-time RT-PCR, loop-mediated isothermal amplification, CRISPR/Cas12a, and serological methods such as ELISA, have been developed in the diagnosis and detection of ToBRFV. Moreover, more than 50 complete genome sequences of different ToBRFV isolates have been deposited in the GenBank database, which provides important sequence information for comparing the specificity and sensitivity of different primers used in RT-PCR. Furthermore, an Expert Working Group will be considered to be organized to prepare a standard diagnosis protocol, which would allow to sharing lots of experience and could be shared for the global benefit.