



**VIRTIGATION – Emerging viral diseases in tomatoes and cucurbits: Implementation of mitigation strategies for durable disease management**

Deliverable Nr. 4.2

Title: Policy document on the role of seed transmission on viral disease spread to Europe (M36, R, PU).

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## 1 PUBLISHABLE SUMMARY

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In this task, we investigated the possibilities for the long-distance dispersal of viruses via seed. We focused our work on the two fast spreading viruses in Europe: the tomato leaf curl New Delhi virus (ToLCNDV) and tomato brown rugose fruit virus (ToBRFV). We investigated both 'seed-borne' and 'seed-transmitted' nature of both viruses. Seed-borne viruses can be present and detected on the seed but will not spread to new crop and thus they pose no threat to the new environment, while seed-transmitted viruses are present on seeds, can be detected and spread to the new environment and thus pose a threat to the new environment it was introduced into.

This document is prepared in two parts; ToLCNDV and ToBRFV separately because of the nature of the viruses being DNA and RNA viruses, respectively, which required different types of investigations. For ToLCNDV, we first investigated the seed-borne nature of the virus by detecting the virus by PCR in four major cucurbit vegetable crops of India such as bitter melon, bottle melon, ridge melon and pumpkin. We found that ToLCNDV was present in varying levels in seeds of different crops from 0% to 6.0% in bottle melon and bitter melon, respectively (over 3500 PCR tests conducted). The virus was present in the embryo of only bitter melon, which raised the possibility of seed-transmitted nature of the virus. Thus, two further experiments were conducted: seed to seedling transmission and grow out tests. In seed to seedling transmission experiments, seeds of all four vegetables were grown in germination papers but found no transmission of the virus from seed to young seedlings by PCR tests. Similar results were obtained in grow out tests on plants grown for two months from infected seeds. Our results agreed with two independent studies from Spain, which also detected ToLCNDV in cucurbit seeds, but the virus was not transmitted to new plants. However, this contradicted some other studies from India, Taiwan and South Korea which have confirmed high levels of seed transmission of ToLCNDV (up to 70%). We therefore support EPPO's conclusion that seed-transmission of ToLCNDV is currently debatable and this is not universal and may be specific to some virus strains and the crop plants they infect. More work is therefore needed before drawing generalized conclusions, and further support EPPO's conclusions on retaining ToLCNDV as a risk for the EU based on its current impact.

We conducted similar experiments on ToBRFV and found that the virus was present on the coats of tomato seeds, embedded firmly between the seed trichomes (hair). The virus was detectable in seeds by ELISA, RT-PCR and microscopic observations. We then conducted extensive grow out tests and found that ToBRFV was seed-transmissible at very low levels of 0.025% (2 infected seedlings out of 8000). These results are similar to earlier studies that also showed low levels of seed to seedling transmission of 0.08%. The PRA concluded that the likelihood of an entry pathway of ToBRFV via tomato seeds was as high and our results support this conclusion. In this document, we have several further recommendations on seed sampling size, sampling rate, and diagnostic tests for minimizing the risk of moving ToBRFV within and into the EU.