

UNECE survey of potato virus testing methods that are associated with seed certification

1. Aim of the virus testing survey

The goal of the UNECE Seed Potato Certification Standard is to act as world reference to facilitate fair international trade by creating a harmonized commercial quality certification system and defining harmonized quality requirements for seed potatoes. The purpose of the UNECE survey of potato virus testing methods that are associated with seed certification was to capture information from around the world. Virus testing is used to support decisions in seed potato certification and the results are of great qualitative and economical value for those who are administering the certification and the farmers using the certified seed potatoes.

The UNECE virus testing survey produced a snapshot of the current practices of virus testing associated with seed potato certification. This information provides a platform for discussion and comparison. The ELISA test method has, for many years, been the most widely used method but in recent years, the number of laboratories using PCR based methods for virus testing has increased. UNECE wish to share the results of the survey of potato virus testing methods with all participating or interested countries.

2. Results of the virus testing survey

The UNECE survey of potato virus testing methods that are associated with seed certification was conducted in 2017. UNECE received 45 responses from certification authorities in 38 countries. More than one response was received from several countries. The following countries completed the survey: Australia, Austria, Belgium, Bulgaria, Canada, Chile, China, Cyprus, Czech Republic, Denmark, Egypt, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, India, Italy, Latvia, Lithuania, Luxembourg, Malta, The Netherlands, New Zealand, Norway, Poland, Portugal, Russian Federation, Slovak Republic, Slovenia, South Africa, Spain, Sweden, Switzerland, UK and USA.

2.1 Laboratory virus testing that is associated with seed potato certification

Thirty certification authorities (70%) responded that potato virus testing was compulsory in their country and a further 11 responded that potato virus testing was compulsory for all crops with exemptions under certain conditions. The most common reason for the exemption was seed category or variety. Virus testing as part of seed potato certification was voluntary for 5 of the certification authorities that completed the survey and 2 authorities stated that virus testing was not done in their country as part of seed certification.

Virus testing was conducted by the same organization as certification for 62% of respondents, other governmental laboratory 17%, private laboratory 26%, university or research institute 24% or laboratory in other country 5%. Typical criteria to choose the laboratory was the efficacy and reliability of virus tests (53%), the price of the virus tests (43%) and the rapidity of the virus tests (40%). 35% responded that they had no possibility to choose the laboratory.

Post-harvest virus testing was the most common method cited by the respondents (45%); however, 43% of those who responded used both leaf testing during the growing season and post-harvest

virus testing. Only 7% stated that they used solely potato leaf testing during growing season for certification.

Potato leaf testing during the growing season was most commonly used (70%) to test the pre-basic category of seed for Potato Virus Y (PVY). PBTC, pre-basic and basic categories were commonly tested for six viruses (PLRV, PVY, PVA, PVS, PVM and PVX) but for the certified category, PLRV and PVY were the most common. Almost all the testing was done using the ELISA method and only a few tests were conducted by PCR. The other viruses (i.e. TSWV, TRV) were more often tested by PCR testing method.

Of those that responded, 93% test basic category seed in their country for PVY by post-harvest virus testing, whether by direct tuber testing, sprouts or leaves in grow out. However, 86% of the certification authorities post-harvest test pre-basic seed for PVY and 80% test for PLRV. Typically, PBTC and pre-basic seed were tested for a range of six viruses (PLRV, PVY, PVA, PVS, PVM and PVX). For basic and certified seed, most certification authorities tested only for PLRV and PVY. On average, approximately 60% of those who responded used the ELISA test method and PCR was used by 20% of respondents. Visual grow out was used for pre-basic and basic seed categories by approximately 5% of respondents and this figure rose to more than 20% for the certified seed category.

2.2 Sampling

The survey responses relating to the sample sizes of leaf testing during the growing season and post-harvest tuber testing were variable. The prevalent sample sizes used for virus testing were 100, 110 or 200 leaves/tubers. The sample size, in some cases, were connected to seed category or area which seed potato was produced.

2.3 Test methods

For PCR based testing, 58% of respondents tested the tuber directly, while 31% used the sprouts for testing. ELISA testing was the most commonly done on leaves grown from tubers in a glasshouse (71%), but sprouts were also used for ELISA testing (36%). Visual assessment of a growing crop (76%) and leaves grown in a glasshouse (50%) were also commonly used methods. When specifically conducting tuber virus testing, the most common methods involved sampling direct tubers (44%) or green sprouts (38%), chitted tubers (eyes open, no green tissue) was used only by 15% of respondents. Often direct testing of tubers was conducted directly after harvest however, some laboratories waited 8 to 12 weeks before testing.

For ELISA testing, commercial kits (77%) were the most widespread (i.e. Bioreba, Loewe, Agdia, Neogen). A total of 61% of respondents pooled samples for ELISA-testing in bulks of 4, 5, 10 or 25 leaves or tubers. For PCR testing, 69% of respondents used methods developed in-house with only 23% of respondents using a commercial kit. Tubers or leaves were pooled/bulked for PCR by 75% of laboratories from 4 to 25 units per individual reaction.

Several published articles provide information on PCR testing of potato viruses. A total of 52% of the respondents stated that the PCR primer sequences for their PVY assay were publicly available

for use. Primer sequences for other viruses were also available (PVX 50%, PLRV 45%, PVA 44%, PVS 42% and PVM 35%).

2.4 Results

The seed certification agencies were asked how the virus testing results are statistically interpreted. The most common method was to use ISTA Seedcalc. The agencies used the laboratory results to determine the classification of the crop using, for example, UNECE standard, EU legislation or National tolerances.

2.5 Quality assurance

A total of 70% of the laboratories used for virus testing were accredited by the National accreditation body and 92% of laboratories had an internal Quality Control system. Of the laboratories surveyed, 59% had validated their PCR virus testing method and 24% reported that validation was in progress. When asked whether the PCR methods used for certification had been independently validated/accredited, 25% answered 'yes' for leaf testing and 31% for tuber testing. Accreditation was usually based on ISO17025 standard. From the survey, 40% of the laboratories participated in ring tests/proficiency tests of potato virus testing by PCR. Ring tests were usually arranged on a national level or with neighbouring countries. It was noted that 45% of seed potato certification authorities audited the laboratory and 32% audited the testing procedures.

Respondents were asked whether ELISA testing on sprouted tubers and direct tuber tests by PCR gave equivalent results. Interestingly, 23% answered "all of the time", 40% "most of the time", 23% "some of the time" and 13% "never". Some of the respondents stated that PCR is more sensitive while some thought that antisera may have a broader range of detection than the PCR primers. The respondents thought that the advantages of testing potato virus by PCR were the rapidity of the tests (70%), sensitivity of the tests (63%) and the efficacy and reliability of virus tests (57%). It is important to note that none of the survey answers supported the claim that the price of the virus tests was an advantage when testing by PCR.

3. Conclusions

Potato virus testing associated with seed potato certification is widely used by all countries producing certified seed. All seed categories are tested in many countries but testing pre-basic and basic categories, and post-harvest testing for PVY and PLRV are the most common. Testing by ELISA is the most widely used virus testing method however there are several laboratories which test potato virus using a PCR based method for certification purposes. Many laboratories used the PCR a based method on a voluntary basis or their validation of the PCR based method was reported to be in progress.

The PCR based methods used for potato virus testing are relatively new and, as such, there are not many commercial kits available. Therefore, it is very important that a proper independent validation of this testing method is done before using them for certification purposes.

UNECE recommends that primers published in scientific journals are used for virus testing associated with seed certification. Certification authorities are encouraged to declare the methods of virus testing officially used to assess the certification of seed lots.

All the results of the UNECE virus testing survey available on the UNECE website:
<http://www.unece.org/index.php?id=50590>