



ISF Viewpoint on Indirect Seed Health Tests

Adopted in Athens, Greece - 29 May 2013

Introduction

The seed industry places a lot of emphasis on seed health. The supply of healthy seeds is essential to help assure growers of a healthy crop. The seed industry works diligently on preventive and hygiene measures, disease inspections, and pathogen control during seed production. The final decision on a seed shipment is mostly based on a diagnostic laboratory test.

The seed health test result is only meaningful if it is a reliable reflection of the risk that is present when the seed lot is brought into the market. The test takes a number of factors including disease dynamics and epidemiology into consideration. The epidemiology of a disease depends upon the pathogen infestation/contamination levels within and/or on the seed, the climate in which the seed is grown, cultural practices (e.g. direct seeding versus transplant production) and the interactions of the host, pathogen and the environment.

A test should be specific to the target pathogen, sensitive, reliable and reproducible. The test protocol should be validated using sound scientific methodology and be publicly available. Ideally, the test should distinguish between the presence of viable and non-viable organisms and/or inactivated genetic material that may be left on the seed after a disinfection treatment. Seed health testing based on these principles will help to ensure that healthy seed is available to growers and prevent the introduction and spread of pathogenic organisms.

Direct and indirect tests

A seed health test in general consists of 3 primary steps: i) isolation of the pathogen from seeds, ii) detection and identification of the pathogen, and iii) confirmation of viability and pathogenicity of the isolate by inoculation of assay plants. With such a **direct** test, the presence of a pathogen on and/or in the seed is demonstrated.

Over the last 3 decades, direct tests have been complemented with faster, simpler and less expensive methods that provide an **indirect** means of checking for the presence of the pathogen. Indirect tests are often very sensitive and rapid, plus they can be performed by anyone with good general laboratory skills. Indirect tests detect proteins (e.g. by Immuno-Fluorescence (IF) and DAS-ELISA) or nucleic acids (e.g. by PCR) that are specific to the target pathogen. As technology progresses, the range of available indirect methods (especially molecular ones) has expanded.

The seed industry embraces these powerful tools and has been actively involved in their development and use for many years. In doing so, it has increased the quality of the seed available to the market place. However, there are also some considerations.

Indirect testing considerations

An inherent drawback of indirect tests is the fact that the presence of viable pathogens is not demonstrated.

In addition, they are able to detect non-viable pathogens as well as closely related, non-pathogenic organisms introducing the risk of false positive results. For a correct interpretation of an indirect test result, it is essential to know and take into consideration specific factors, such as variability in the proteins and/or genetic material of the pathogen and its similarity to closely-related non-pathogenic organisms.

Thirdly, an indirect test does not clearly define the biological relevance of a positive test result. Therefore, it is imperative that the indirect test undergoes proper validation before being used. Objective evidence should be presented showing that the indirect test fulfills the intended purpose. Ideally, test validation data should be publicly available in peer reviewed scientific literature.

Assessment of indirect test results

If an indirect test yields a 'negative' result then no confirmatory test is necessary.

If an indirect test yields a 'positive' result then a second, direct test that confirms pathogenicity is crucial in order to obtain a reliable, conclusive positive test result.

If, however, there is no direct test available, then the seed industry recommends a confirmatory indirect test that is based on different biological principles, such as a serological test (IF, DAS-ELISA) to confirm a result obtained using a molecular technique (PCR).

Recommendations

It is the opinion of the seed industry that:

- A positive result of an indirect test should be considered as preliminary and should always be followed with a confirmatory test that is preferably a direct test.
- Diagnostic protocols used for seed health testing as well as the seed test method validation results should be publicly available.