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Articles in this issue

Molecular diagnostic methods can prevent unnecessary rejection of organic seed lots

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In some years, large amounts of organic seed lots are discarded due to the presence of high levels of seed-borne diseases. In barley it is especially the presence of the fungal species *Pyrenophora teres* (barley net blotch) and *P. graminea* (barley leaf stripe, photo 1), which causes rejection of seed lots.

Photo 1. Barley leaf stripe.

Thresholds for seed infection

The threshold for the presence of *Pyrenophora* species is at present 5 pct. infected seeds. This is a combined threshold for both *P. teres* and *P. graminea* due to the fact that the conventional seed health test currently used cannot distinguish between these two species. The threshold for *P. teres* alone is 15 pct. However, only an additional test in the greenhouse for leaf symptoms can tell how much *P. graminea* is present. This test, however, needs at least one month for completion. As a result most seed lots are tested without differentiation between *P. teres* and *P. graminea*. Consequently, the 5 pct. tolerance level shall apply.

Since *P. graminea* is less frequent than *P. teres* in barley seed lots there is no doubt that seed lots are unnecessarily rejected. Introduction of a DNA-based seed health test for detection of *Pyrenophora* infection in barley seeds can reduce the number of rejected barley seed lots.

DNA-based methods for *Pyrenophora graminea*

In contrast to conventional seed health tests, DNA-based methods often have the advantage of being specific to the species level, sensitive and rapid with the potential of being automated.

The DNA-based method polymerase chain reaction (PCR) can be used to identify and detect a specific pathogen in plant tissue and provides a rapid test. Details of the PCR method is presented in **Box 1**.

In the **ORGSEED project** one aim has been to develop a fast method for detection and quantification of *P. graminea*. A real-time PCR method for the specific detection of *P. graminea* in seeds has been designed from a DNA sequence that is unique for *P. graminea*.

Results presented in **Figure 1** and **Figure 2** illustrates the specificity and standardisation of the test method.

Correlation between the amount of *P. graminea* DNA and the level of infected seeds

Samples of harvested seeds from a field trial infected with *P. graminea* have been used to test the correlation between the amount of *P. graminea* DNA and the infection level. The samples were all from the same spring barley variety 'Agneta'.

The seeds were tested with the blotter method, in which the seeds are

screened for the presence of *Pyrenophora* conidia after 7 days of incubation. As this method cannot differentiate conidia of *P. graminea* and *P. teres* the method may overestimate the level of *P. graminea* infection. However, it is assumed that the level of *P. teres* infection is constant in the samples as they were all from the same field trial and the same variety.

The samples were also tested in the greenhouse where plants with leaf stripe symptoms are counted 4-5 weeks after sowing. The samples were tested with the real-time PCR method and the amount of *P. graminea* DNA was quantified. As an endogenous control a test for a barley gene was performed in parallel to normalise for poor DNA extraction and PCR inhibitors.

The amount of *P. graminea* DNA in a sample of 200 seeds is significantly correlated with the number of infected seeds tested by the blotter method (R^2=0.68, P<0.001) as shown in **Figure 3**. The amount of *P. graminea* DNA is also significantly correlated with the number of infected plants in the green house test (R^2=0.55, P<0.001) despite of some large variations among samples **(Figure 4)**.

The infection level of individual seeds may vary considerably causing variation in the amount of DNA among seeds within a sample. This is a weakness when trying to establish a relationship between the total amount of *P. graminea* DNA and the percentage of infected seeds or plants in a sample.

The PCR is amplifying DNA regardless of the viability of the fungal cells meaning that PCR will also measure DNA from fungal tissue, which will not produce conidia on the seeds or cause symptoms on the leaves. This may be the reason why some samples giving 0 percent infected plants show the presence of P. graminea DNA when tested with PCR.

Future use of the *P. graminea* test

The possibility to distinguish *P. graminea* and *P. teres* will allow to make use of individual thresholds for the two diseases thereby preventing unnecessary rejections of seed lots having less than 15 percent of infection with *P. teres*.

Real-time PCR is an extremely sensitive method. It has been demonstrated that seed infection levels as low as 0.5 percent, which is the detection limit for the blotter test and the green house test, can be detected using the PCR-method.

A significant correlation between the amount of *P. graminea* DNA measured by PCR and the infection level can be established. However, the wide variation among samples may imply that the PCR cannot be used as the only test for all seed lots. Yet, it can be used to establish lower and upper thresholds of DNA content, and in a preliminary test of a sample it is then possible to see if the DNA content is below or above these thresholds.

A major advantage of the method is its speed. A sample can be tested within a few hours in contrast to 7 days (blotter test) or 4-5 weeks (greenhouse test).

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