Sammanställning av slutrapportering

Bestämning av förekomst av patogena svampar i vete med PCR-teknik

Resultaten från projektet har sammanställts i en slutrapport från avdelning för precisionsodling ”Bestämning av förekomst av patogena svampar i vete med PCR-teknik. Avdelningen för precisionsodling. Rapport 18, Skara 2008.
Tillgänglig via länken: http://pub-epsilon.slu.se/292/01/porapp18.pdf

Resultaten har också presenterats i Sverige på

- Växtskydds- och växtodlingsdagar i Linköping den 11-12 december 2007
- Regional växtodlings- och växtskyddskonferens, Uddevalla 10-11 Jan 2008

Internationellt har resultaten presenterats med två posters på den 9th International Congress of Plant Pathology, August 24-29, Turin, Italy. (bifogade)

Topic area: Molecular diagnostics for plant pathology
Comparsion between real-time PCR and ocular grading of wheat pathogens
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Topic area: Molecular diagnostics for plant pathology
Quantitative real-time PCR - an effective tool for assessment of fungal flora in field trials
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Två manuskript kommer också att lämnas in oktober 2008 för peer view publicering i Pest Managemant Sciences (1) och European Journal of Plant Pathology (2)

1. **Assessment of wheat leaf spot diseases in field testing of cultivars and fungicides**
Lerenius C , Almquist C and Jonsson A

2. Quantitative real-time PCR in comparison with visual grading for assessment of fungal infection of *Septoria tritici, Stagonospora nodorum* and *Drechslera tritici-repentis* in winter wheat
Almquist C , Filipsson C, Lerenius C and A. Jonsson

Skara den 1 september 2008

Anders Jonsson

Comparison between real-time PCR and ocular grading of wheat pathogens

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Introduction

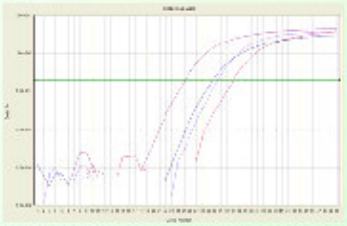
The detection and quantification of the wheat pathogens *Septoria tritici*, *Drechslera tritici-repentis* (*DTR*) and *Stagonospora nodorum* has so far mainly relied on a visual inspection of infected leafs. This procedure is both time consuming and requires experienced personnel. Faster, more accurate and reliable methods suitable for routine diagnosis are therefore needed. The objective of the present study was to develop real-time PCR assays for quantification of these three plant pathogens and to correlate the amount of pathogen DNA to the results from a visual disease grading.

Material and methods

More than 300 winter wheat samples were collected from field experiments in southern and central Sweden during 2006 and 2007. The dominating plant pathogens were determined by a visual inspection and reported as percentage infected leaf area.

DNA was extracted from the wheat samples using commercial kits for plant DNA extraction. Newly designed primers and probes and a previously published primer-probe set were used to specifically detect each pathogen using quantitative real-time PCR (qPCR). The amount of plant pathogen DNA was determined using relative quantification (comparative C_T method, $2^{-\Delta\Delta CT}$, Fig. 1) and a wheat-specific gene was used as reference.

A



B

$$\Delta Ct_u = Ct_{u,pathogen} - Ct_{u,wheat}$$

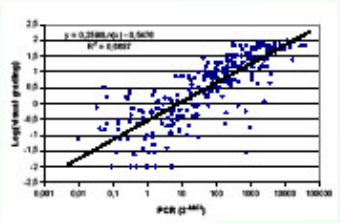
$$\Delta Ct_c = Ct_{c,pathogen} - Ct_{c,wheat}$$

$$\Delta\Delta Ct = \Delta Ct_u - \Delta Ct_c$$

$$\rightarrow 2^{-\Delta\Delta CT}$$

Figure 1. A: Amplification plot showing the detection of the three pathogens and the reference gene (wheat) in the same wheat sample. B: Comparative C_T method. u: unknown sample and c: control sample. $2^{-\Delta\Delta CT}$ is the amount of pathogen normalized to wheat and relative to the control sample.

A



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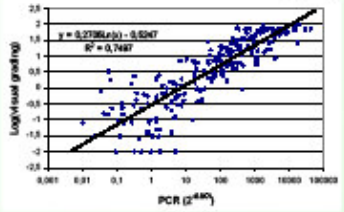

Figure 2. Correlation between visual grading (%) and qPCR for *S. tritici* at several different sites in Sweden during 2006 and 2007. Figure A shows the result for all samples and B shows the result for samples infected with *S. tritici* but with less than 5% infection of the other two pathogens (*DTR* and *S. nodorum*).

Results and Discussion

The developed methods showed high specificity, high repeatability (relative SEM of 10, 8 and 18%, respectively, n=2) and high reproducibility between laboratories. A good correlation (R^2= 0.67 for *S. tritici* and R^2= 0.51 for *DTR)* was observed between visual grading and real-time PCR determinations for all wheat samples taken during 2006 and 2007 (Fig. 2), especially when one pathogen dominated on the leafs (R^2= 0.75 and R^2= 0.59).

Although visual checks often indicated the presence of only one fungus, qPCR results showed that all three pathogens were present in the majority of samples. However, *S. nodorum* was very seldom recorded in the visual grading.

Comparison of the number of genomes in the control samples indicates that $2^{-\Delta\Delta CT}$ might be compared directly between pathogens. This is confirmed by the high similarity between correlation equations for visual grading vs. PCR for both *DTR* and *S. tritici* (Fig. 2 and 3).

Even pre-symptomatic infections were detected using qPCR. $2^{-\Delta\Delta CT}$-values for presence of fungi, growth of fungi and visual symptoms, might be proposed. For example: *S. tritici*: 0 = free; <0.05 = presence of fungus, no growth or visual symptoms; 0.05-0.5 = growth, but no significant leaf spots; 0.5-1000 = significant leaf spots; >1000 = heavy infection with more than 10-20% infected leaf area.

Conclusions

qPCR is an excellent method for grading of fungal diseases in wheat with good correlation to visual grading. Pathogens present in low amounts can be detected with high specificity.

The values from the comparative C_T method, $2^{-\Delta\Delta CT}$, can be used to compare levels of fungal infections between fields.

The qPCR methods make it possible to detect pre-symptomatic infections in field samples, mainly for *S. tritici* but to some extent also for *DTR*.

A classification of an invading pathogen is suggested based on the $2^{-\Delta\Delta CT}$-values.

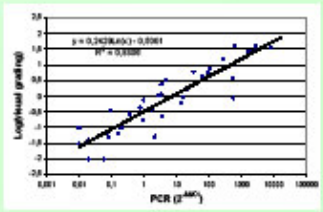

Figure 3. Correlation between visual grading and qPCR for *DTR* at Kilsgården (2006 and 2007).

This work was funded by the Swedish Farmers' Foundation for Agricultural Research.

Quantitative Real-Time PCR
- an effective tool for assessment of fungal flora in field trials

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Introduction

Septoria tritici, *Drechslera tritici-repentis* (*DTR*) and *Stagonospora nodorum* are three of the most common pathogens on winter wheat in Sweden. Real-time PCR has made it possible to detect and quantify these pathogens in a more accurate and objective way compared to traditional visual grading estimating the infected leaf area. In this project, quantitative real-time PCR (qPCR) assays are used to study the effect of different fungicides and to assess the fungal flora in different wheat cultivars.

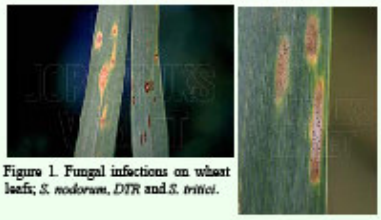

Figure 1. Fungal infections on wheat leafs; *S. nodorum*, *DTR* and *S. tritici*.

Material and methods

Over 300 wheat samples were collected from field trials with fungicide and cultivar testing in southern and central Sweden during 2006 and 2007. Several different fungicides were used, both a strobilurin (pyraclostrobin, Comet) and triazoles (prothioconazole and propiconazole, Proline and Tilt). The cultivars tested were: SW Harnesk, Mon Olivin, PBIS Florett and LP Skalmeje.

The dominating plant pathogens were identified by visual inspection at sampling, and level of infection was recorded as percentage visibly infected leaf area. Wheat samples were analysed using the developed qPCR assays. The amount of pathogen DNA was determined using relative quantification (comparative CT method, $2^{-\Delta\Delta CT}$) and a wheat specific gene was used as reference.

Results and discussion

The qPCR assays were used to study the effect of fungicides on the fungal flora. A very small variation between replications was observed (Figure 2). Using qPCR it was possible to detect significant fungicide effects at low infection levels (<10%). For example, the fungicide effect on *DTR* at both Vattholma and Rallatorp was significant (Fig. 2, Duncans Multiple Range Test).

A good correlation was observed between qPCR and visual grading of the diseases (Figure 3).

qPCR was also a useful tool for quantifying the amount of the three wheat pathogens in field trials where different cultivars were evaluated (Figure 3 and 4). At Russelbacka, all three pathogens were detected using qPCR. However, the visual grading only showed high levels of *S. tritici* (10-60%) and low levels of *DTR* for Florett and Skalmeje (<0.2%).

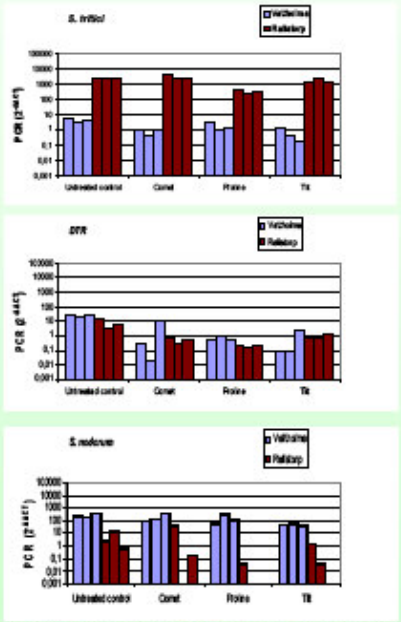

Figure 2. Results from the qPCR analysis of the three pathogens at Vattholma and Rallatorp (Sweden). PCR results are shown for three repetitions of the untreated control and three different fungicides (Comet 0.5 l/ha, Proline 0,4 l/ha and Tilt 0,25 l/ha). Two DNA extractions were made for each repetition.

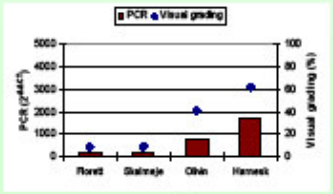

Figure 3. Results from both qPCR and visual grading of *Septoria tritici* at Russelbacka (Sweden). The diagram shows the results for four different wheat cultivars.

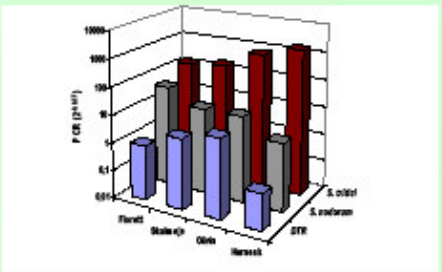

Figure 4. Results from the qPCR analysis of the three pathogens at Russelbacka (Sweden). PCR results are shown for four different wheat cultivars.

Conclusions

qPCR has proven to be a valuable technique for an objective detection and quantification of wheat pathogens and can be used both for fungicide and cultivar evaluation.

In cases where several pathogens are present in the canopy, visual grading can be misleading since the symptoms of the dominating fungus may conceal symptoms caused by others. The new qPCR assays also make it possible to easily distinguish fungal infections from physiological spots.

This work was funded by the Swedish Farmers' Foundation for Agricultural Research.