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Identifying resistance genes in wheat against common bunt (*Tilletia caries*) by use of virulence pattern of the pathogen

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Abstract

455 wheat varieties and breeding lines were grown in the field, contaminated with 7 to 11 different races of common bunt. Based on the reaction of the lines to the different virulence races, it was possible to group the lines by differential varieties with known resistance genes, indicating that they may have one or two of the resistance genes *Bt1*, *Bt2*, *Bt5*, *Bt7*, *Bt13*, *BtZ* or Quebon-resistance. Based hereof, genetic markers will be developed using a genome-wide association study (GWAS).

Keywords

Genome-wide association study · LIVESEED · marker assisted selection · organic plant breeding · seedborne disease · *Triticum aestivum*

Introduction

Wheat can be infected by the seed and soilborne diseases common bunt (*Tilletia caries*, *T. laevis*), and dwarf bunt (*T. contraversa*), but it has long been known that different varieties have different susceptibility to the disease (Tscharner 1764, Kühn 1880, Tubeuf 1901, Cobb 1902, Hecke 1906 & 1907, Pye 1909, Darnell-Smith 1910, Kirschner 1916). During the past century, a number of specific resistance genes have been identified (Hoffmann & Metzger 1976, Goates 2012). These resistance genes are common for the three pathogens and are present in a set of differential varieties, that can be used to describe the virulence pattern in bunt populations. These differential lines, however, are not well adapted to modern agriculture in Europe. On the other hand, a number of adapted resistant European wheat varieties are known, but often it is unknown which resistance genes are causing their resistance. Therefore, wheat breeding aiming to introduce bunt resistance into modern adapted material needs to base the breeding either on unadapted lines or adapted lines with unknown resistance genes.

The bunt pathogens is divided into different virulence races, each able to infect plants with different resistance genes (Hoffmann & Metzger 1976, Goates 2012). Often bunt spore collections are mixtures of different races. When a wheat variety is infected with a mixture of races, and reinoculated with spores from this infection, the infection level often raises because of selection of virulent races within the mixture (Weston 1932, Bever 1939). Since 2010, Agrologica has worked on purifying bunt races, that are homogeneous in their ability to infect plants with certain resistance genes (Borgen 2015). By infecting wheat varieties with a range of races of common bunt with different virulences towards the resistance genes, it is possible estimate which resistance gene they have.

To improve the basis for bunt resistance breeding, the LIVESEED project has initiated a research program that will try to identify resistance genes in adapted varieties and breeding lines. Later, based on the results of this identification, the project will develop genetic markers for the resistance genes.

Material and methods

Before sowing of winter wheat in 2017, 450 wheat varieties and breeding lines that have demonstrated resistance to common bunt in previous trials were contaminated with 7 different virulence races of common bunt; 62 of the lines were also contaminated with additionally 4 virulence races. The spores used to infect the lines, all originate from Denmark. Nielsen (2000) collected spores from different places in Denmark and bulked them into a mixed population. This bulk population was used to infect a range of varieties (Steffan 2014). Spores from infected heads of resistant varieties were collected, maintained and multiplied on these varieties to confirm virulence against the resistance gene in question (Borgen 2015, 2016).

The tested wheat lines were selected in order to cover differential lines with the known resistance genes, and a balanced amount of lines with each of 7 resistance genes that were aimed to be identified in the study. The resistance genes include *Bt1*, *Bt2*, *Bt5*, *Bt7*,

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Bt13 and *BtZ*. The genes were selected based on the virulence of bunt races.

About 50 seed were sown with each line in each treatment. After heading, the plants were scored for visible symptoms of infection in the head.

Results and discussion

The infection rates of 180 selected wheat lines are presented in Fig. 1, where the infection is colored based on infection level. The lines are sorted based on a subjective evaluation of similarity in reaction to the different virulence races. Infection of lines with zero infection to all races are not presented and are hypothesized to carry multiple genes with additive effects.

The infection rates ranged from 0 to 100% infection with some lines being resistant to all virulence races, and others being susceptible to all races.

Line PI 181463 (Thule III) and a few lines derived from crosses with this line were infected with at a low rate when contaminated with race Vr13. This is surprising, since this race demonstrated high virulence against *Bt13* in previous years. This may indicate that the spores in 2017 may have been of low vitality and/or been applied in low quantity.

Most differential lines were infected by one or more of the virulence races. This shows that virulence is present against most of the known *Bt* resistance genes. However, lines with *Bt9* or *Bt11* were not infected by any of the races (data not presented). It is inconclusive if lines with *Bt4* were infected or not, since two lines are listed to have *Bt4*, and one was infected and the other not. This may be due to heterogeneity within the differential lines (Dumalasova, pers. commun.).

Bt12 was infected with race 341 (data not presented), which is surprising since virulence to *Bt12* has not previously been described in Europe. Also *Bt6* was infected by this race, which is also surprising, as *Tilletia leavis* has never been observed in Denmark, and virulence against *Bt6* has so far only been observed in Eastern Europe in areas where *T. leavis* is present (Mascher *et al*. 2016). However, race 341 has not been identified at species level.

Based on the sorting presented in Fig. 1, it seems that some lines react in a similar way to the different races, and it is hypothesized that lines with similar reaction have the same resistance gene. Since each group has one or more differential lines with known resistance genes, it is hypothesized that the groups represent lines with the same resistance genes as the differential line in the group. However, *Bt10* and *BtZ* react in a similar way to the different races. Differential lines with *Bt10* and *BtZ* has been assessed with the genetic marker identified for *Bt10* (Laroche *et al*. 2000), and only *Bt10* had this marker which supports the fact that *Bt10* and *BtZ* are indeed two different genes and that race 10 in this study apparently is virulent to both *Bt10* and *BtZ*. Therefore, the distinction between *BtZ* and *Bt10* in this study is based on information about the parents.

In this study, lines with multiple resistance genes were not infected by any of the races. However, some combinations of dual resistance are relatively easily overcome by the development of new virulence races of the pathogen, given that virulence against the parent resistance gene are present in pathogenic races in the region (Hoffman 1982). Therefore, a safer strategy is to combine resistance genes where virulence against at least one of the genes are rare. In Europe, virulence is frequently found against *Bt7* and relatively common also against *Bt1*, *Bt2* and *Bt5*. Our study shows that these genes are also found in several commercial varieties in Europe, and this is likely the reason for the virulence. Using these resistance genes alone can, therefore, not be used as the only strategy to control the disease, but must be combined with other control measures or at least be followed with seed analysis for the presence of spores prior to sowing.

We believe that this study can be used as a data foundation for a GWAS to identify genetic markers for the bunt resistances in question. The trial will be repeated in 2018/19 to confirm the results and this hypothesis further pursued within the LIVESEED project.

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References

Bever WM (1939) Reinoculation of resistent varieties of wheat with purified physiologic races of *Tillitia tritici* and *T. levis*. Phytopathol 29:863-871.

Borgen A (2015) Purifying virulence races of common bunt (*Tilletia caries*) to identify resistance genes in wheat. Program and abstracts of the COBRA final conference, 24-25 Nov, Vingsted, Denmark, pp 32-34.

Borgen A (2016) Screening wheat varieties for resistance with purified virulence races of common bunt (*Tilletia caries*). In: Morgounov A, Muminjanov H (eds), XIX International Workshop on Smuts and Bunts, 3-6 May, Izmir, Turkey, Book of Abstracts, pp 27-30.

Cobb NA (1902) Comparative observations on the brush of fifty varieties of wheat. The Agricultural Gazette of New South Wales 13:647-649.

Darnell-Smith GP (1910) Observations upon the disease of wheat known as "bunt". Report of the Government Bureau of Microbiology for the Year 1909, pp 64-69.

Goates BJ (2012) Identification of new pathogenic races of common bunt and dwarf bunt fungi, and evaluation of known races using an expanded set of differential wheat lines. Plant Dis 96:361-369. DOI: 10.1094/PDIS-04-11-0339

Hecke L (1906) Die Brandkrankheiten und ihre Bkämpfung. Wiener landwirtschafliche Zeitung 56 (33):318-319.

Hecke L (1907) Die Triebinfection bei Brandpilzen. Zeitschrift für das landwirtschaftliche Versuchswesen in Österreich 10 (6):572-574.

Hoffmann JA (1982) Bunt of wheat. Plant Dis 66:979-986. DOI: 10.1094/PD-66-979

Hoffmann JA, Metzger RJ (1975) Current status of virulence genes and pathogenic races of the wheat but fungi in the northern USA. Phytopathol 66:657-660.

Kirschner O (1916) Über die verschiedene Empfänglichkeit der Weizensorten für die Steinbrandkrankheit. Z Pflkrankh 26:17-25.

Kühn JG (1880) Beobachtungen über den Steinbrand des Weizens. Österreichisches landwirtschaftliches Wochenblatt 6:1-2

Laroche A, Demeke T, Gaudet DA, Puchalski B, Frick M, McKenzie R (2000) Development of a PCR marker for rapid identification of the *Bt-10* gene for common bunt resistance in wheat. Genome 43:217-223. DOI: 10.1139/g99-113

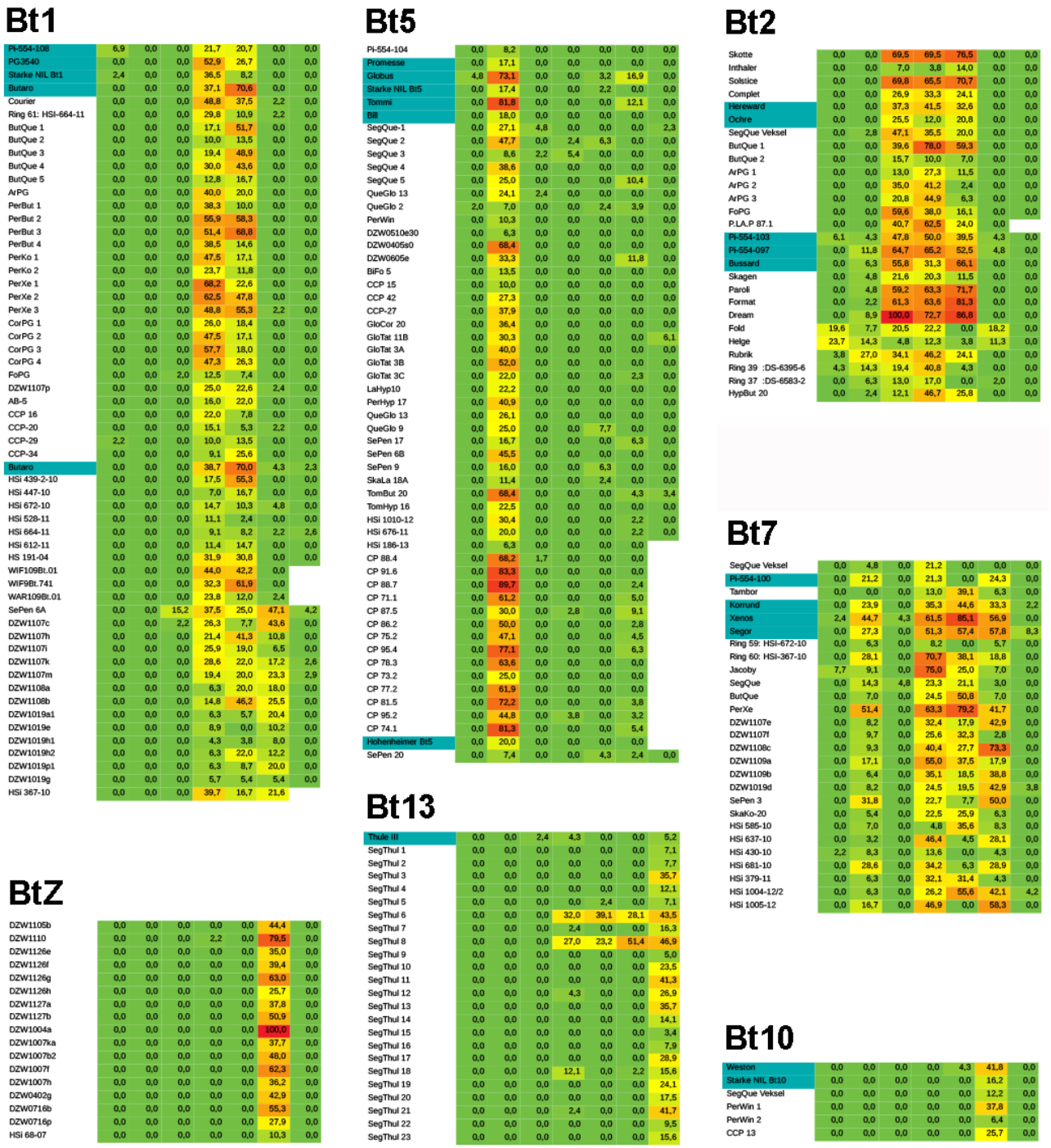

Figure 1: Infection of 180 selected wheat varieties and breeding lines contaminated with spores of 7 different virulence races of common bunt (*Tilletia caries*). The colour gradient indicates infection level from 0% (green), 20% (yellow) to 100% (red). The lines are grouped based on the similarity in reaction to the different races. Lines with similar reaction as differential lines with known resistance genes are hypothesized to carry the same resistance gene, supported by knowledge of the parents.

Mascher F, Borgen A, Dumalasova V, Müller K-J, Hole D, Dell'Avo F, Liatukas Ž, Müllner AE, Henrikson T, Pregitzer A, Al-Maroof EM, Morgounov A (2017) Multilocal resistance assessment against common bunt of wheat (*Triticum aestivum*). 67. Jahrestagung 2016, 21-23 Nov, Raumberg-Gumpenstein, pp 37-38. Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs.

Nielsen BJ (2000) Resistens mod stinkbrand i vinterhvede. In: Deneken G, Pedersen JB (eds), Sortsforsøg 2000. Korn, bælgsæd og olieplanter, pp 12-13. Landbrugets Rådgivningscenter, Århus, Denmark.

Pye H (1909) Diseases and pests of cereals. The Journal of theDeptartment of Agriculture, Victoria 7 (6):368-373.

Steffan PM (2014) Biotechnology assisted wheat breeding for organic agriculture. Phd thesis, Univ Copenhagen, Frederiksberg, Denmark.

Tscharner NE (1764) Von Brand und von dem Rost im Getreide. Abhandlungen und Beobachtungen durch die Ökonomische Gesellschaft zu Bern 5:25-40. DOI: 10.5169/seals-386606

Tubeuf, C von (1902) Weitere Beiträge zur Kentniss der Brandkrankheiten des Getreides und ihre Bekämpfung. Arbeiten aus der Biologischen Abteilung für Land- und Forstwirtschaft am Kaiserlichen Gesundheitsamte 2:437-467.

Weston WARD (1932) The relative resistance of some wheat varieties to *Tilletia caries* (DC.) Tul. (=*T. tritici* (Bjerk.) Wint.). Ann Appl Biol 19:35-54. DOI: 10.1111/j.1744-7348.1932.tb04305.x