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Plant-microbe interactions

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Resistance to toxigenic fungi in cereals: the major toxin regulating agent

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Abstract

Various studies have shown that toxin regulation is connected in most cases to the resistance. Therefore, plant breeding for resistance is the most important tool to reduce toxin contamination. We should be also aware that genotypes with significant toxin overproduction exist which pose an additional risk to food or feed safety. A considerable variation - 10 fold or higher differences - exists in the resistance between cultivars. Hence, the first step is to identify from the registered cultivars those that represent a low food and feed safety risk. Generally, resistance and grain yield level should not be contradictory: we have high grain yields in resistant and susceptible genotypes. Having enough data over years, the breeders will be able to find and breed genotypes with higher resistance, better inbreds and better hybrids. Registration and post registration tests are inevitable. Otherwise, highly susceptible cultivars or hybrids may be produced on our fields. For screening, artificial inoculation methods are needed. Moreover, integrated pest management, better agronomy, and better soil conditions with high microbial activity have to be exploited. The use of fungicides may be effective in small grain cereals, however, in maize the use of fungicides is hitherto not common. In this case, still more research is needed. Resistant cultivars are necessary as they are also easier to protect. Therefore, the production of grains under epidemic conditions will need both higher resistance levels and a better control by fungicides.

Keywords *Aspergillus flavus* · ear rot · Fusarium head blight · mycotoxin · *Triticum aestivum* · *Zea mays*

Introduction

In Europe, all cereals are jeopardized by epidemics caused by toxigenic fungal diseases. Toxin contamination is normally linked to epidemics indicating a relationship between toxin contamination and severities of epidemics. However, in small grains experimental data did not always support the close relationship between epidemics and toxin contamination. As analyses showed, natural epidemics didn't infect nurseries uniformly: genotypes flowering at conditions unfavorable for the disease expressed pseudo-resistance. As weather conditions are usually not stable for a period of one month, data for different inoculation dates might be very different and stability of a genotype's response not be granted. Many pathogens can cause Fusarium head blight (FHB) epidemics, but the main *Fusariu*m species is *F. graminearum*. Resistance to this species was found to be the same as for other *Fusarium* spp. (MESTERHÁZY *et al*. 2005, 2007). In this case the control of deoxynivalenol (DON) was enough, no other toxins were found in significant amount. Toxin contamination is also influenced by different genetic actors, *e.g*. the production of masked mycotoxins such as DON-3-glycoside (LEMMENS *et al*. 2005), characteristic to the 3BS QTL, but also other QTL were responsible for that. Recently, LEMMENS *et al*. (2016) showed that masked DON is actually present in each cultivar, however, the more resistant cultivar contained less DON. Masked DON seems to be a danger close to the permitted contamination. SZABÓ-HEVER *et al*. (2014) showed that in 'Frontana' QTL were identified which influence (i) only FHB visual scores, (ii) FHB scores and Fusarium damaged kernel rate (FDK) together, (iii) FDK and DON content, and (iv) FHB, FDK and DON, indicating a rather complex genetic regulation. In our tests FHB, FDK and DON were regularly measured and generally significant correlations were determined between DON and FDK (MESTERHAZY *et al*. 1999, 2005, 2011, LEHOCZKI-KRSJAK *et al*. 2010).

In maize we have much less data, and the variance in the toxin data is considerable. Correlations between severity and toxin contamination are seldom higher than medium. The problem is more complicated than in wheat. Resistance to different species (*F. graminearum, F. verticillioides, Aspergillus flavus*) is not linked automatically (MESTERHAZY *et al*. 2012), but own results support the findings reported in the literature. *A. flavus* is a special case as

Mesterházy A, György A, Szabó-Hevér A, Varga M, Lehoczki-Krsjak S, Szabó B, Varga J, Bagi F, Tóth B (2017) Resistance to toxigenic fungi in cereals: The major toxin regulating agent. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed.), 67. Jahrestagung 2016, 21-23 November, Raumberg-Gumpenstein, pp 1-3. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-45-9

aflatoxin was considered a storage problem, but now it is slowly accepted that in epidemic years (*e.g.* 2007, 2012) field contamination by aflatoxins is not an exception.

Material and methods

The methodical background for wheat is described in detail in MESTERHAZY *et al*. (2015). In maize, resistance tests were carried out with 20 hybrids in 2012 and 2013. The plant material was tested against four toxigenic species (*F. graminearum, F. culmorum, F. verticillioides, A. flavus*). Data were expressed in percentage of fungi coverage of the ears.

Results and discussion

Wheat. The tests of the last years proved for wheat that best differentiation of the genotypes was achieved by the spraying method with 48 h coverage of the heads by polyethylene bags (MESTERHÁZY *et al*. 2015). Spraying inoculation combined with mist irrigation and the spawn method gave significantly lower data for all traits. With respect to the traits the visual scores were the least reliable for breeding purposes, FDK was significantly better. In the four years' test with 40 genotypes we found a significant correlation between DON and FDK (*r*=0.81, *P*=0.001). We identified six genotypes with DON overproduction, *e.g*. they produced significantly more DON than could be supposed based on the visual rating. This aspect was not analyzed earlier as we looked for resistance, but from the food safety aspect it is important as these genotypes show higher food safety risks than genotypes with regular reaction. Two genotypes were found with significantly lower DON production as supposed. In these two cases we can think about masked DON of different types, but also other mechanisms can be present. This means that 20% of the genotypes reacted diversely. As this may cause food safety problems careful toxin analysis can't be avoided. It is considerable that visual scoring data correlated less with DON contamination (*r*=0.51, *P*=0.001) which supports many earlier findings. The study verified that resistance is the most powerful DON regulating agent. For this reason the DON problem is mainly a breeding problem and breeding task. We should breed for low FDK, this is enough for the regular breeding work, but before submitting the lines to VCU and DUS trials, a toxin test is inevitable. Toxin analyses should be also made during the registration trials including resistant and susceptible check cultivars to evaluate the risk of the new variety candidates. For breeding, artificial inoculation is suggested as most of the described resistance QTL are not validated and their interactions, with some exceptions, are yet unknown.

How is the screening process made? The prerequisite of efficient selection is the careful planning of the crosses. At best both parents have at least medium resistance. The first artificial inoculation is applied in F_3 when 10-15 heads of a good looking line will be inoculated with two inocula. In 'B lines' (first yield test on 5 m^2 plots), separate rows are sown and inoculated as described before. In 'C lines', four inocula are used in single row plots, their mean performance provides the entry value. In the multilocation test two replicates are made, now in two-row plots. Earlier we measured DON for all inocula, for serial work we pool the ear samples of the four inocula (2-2 of each inoculum), and only one DON measurement will be made. In this test yearly 90-150 genotypes are tested, check cultivars, and significant competitor cultivars are also included. In 2011, 145 genotypes were tested. The correlation between FHB and FDK was *r*=0.63 (*P*=0.001), between FHB and DON *r*=0.58 (*P*=0.001) and between DON and FDK *r*=0.75 (*P*=0.001) (Figure 1).

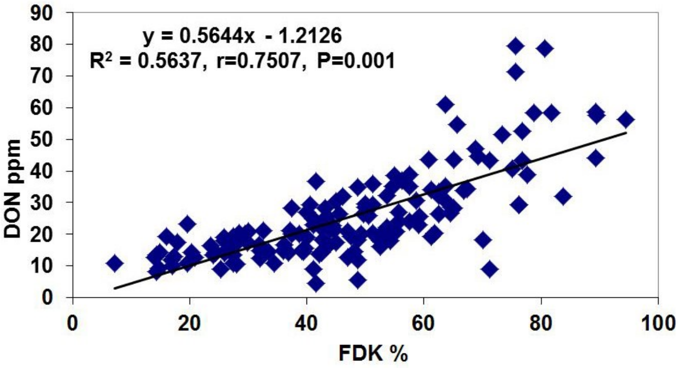

Figure 1: Regression between the percentage of Fusarium damaged kernels (FDK) and the deoxynivalenol (DON) concentration in the multilocation trial 2011 (*n*=145)

This means that in a single year we get high and significant correlations, but differences between years may be considerable. Therefore, genotypes with low FDK and low DON can be determined more reliable. For practical breeding nobody will select material with high FDK independently of DON. In our population we identified good resistance in genotype that did not have known FHB resistance. Therefore, screening of breeding material can identify good and adapted sources of FHB that could be useful for growing in the field and for further crosses.

Maize. *F. graminearum* and *F. culmorum* revealed good agreement (*r*=0.76, *P*=0.001) as they did 30 years ago (MESTERHÁZY 1982). Their correlations with *F. verticillioides* were less close, but significant (Fg/Fv *r*=0.71, *P*=0.001; Fc/Fv *r*=0.65), but no common resistance could be established with *A. flavus* (*r*=-0.01-0.31). Other tests later proved that the correlations between *F. graminearum* and *F. verticillioides* can be not significant, but *A. flav*us seldom was connected to any of the *Fusarium* resistances. This meant for us that generally the resistance background is not common, so every resistance should be determined parallel. In spite of this we found in 'SzeTC465' an excellent resistance level at a variance of 1.7 to the different pathogens at ranks between 1 and 4. The highest variance was 45.3 where ranks varied between 2 and 17 from the 20. We identified several very susceptible hybrids to all pathogens with a low variation of 4.0 and ranks between 15 and 20. The difference between hybrids is normally 10-fold or higher, so we have the chance to identify low risk hybrids for feed safety. The toxin relations are less clear than in wheat. However, the tendency is similar. For the majority of hybrids the toxin contamination is proportional with the ear symptom coverage, but toxin overproduction can be 2 to 3-fold compared to enotypes with proportional toxin production. In Figure 2 the correlation between the percentage of *F. graminearum* coverage of ears and the DON concentration is shown. Three genotypes are marked with light blue that have 100-300 mg/kg, the regular response is between 40 and 60 mg/kg. When the three DON overproducers are not considered, the correlation is *r*=0.72 (*P*=0.01). We have similar experience with fumonisins and aflatoxin B1. On the other hand, we can identify genotypes that have low infection severity and low toxin production. They are what we need. This is important as for maize the measurement of toxin production is even more important as for wheat, due to higher concentrations.

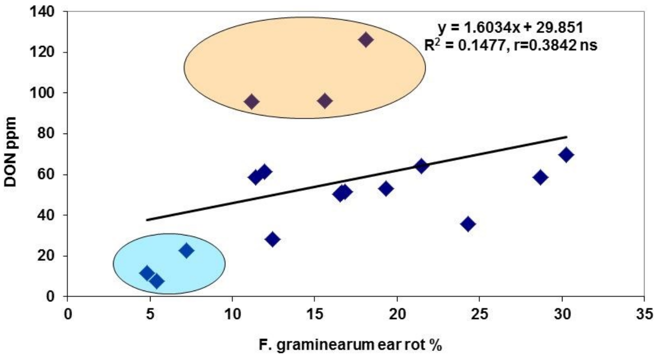

Figure 2: Regression between *F. graminearum* ear coverage from artificial inoculation and DON contamination in maize test 2013.

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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Effect of wheat resistance breeding on masked and non-masked *Fusarium* mycotoxins

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Abstract

The most important threat associated with Fusarium head blight (FHB) is the possible mycotoxin contamination. *Fusarium* spp. can produce a range of different mycotoxins, among which deoxynivalenol (DON) is most common. Metabolisation of mycotoxins *in planta* yields so called ‘masked’ mycotoxins, which are not routinely analyzed, but remain hazardous since endogenous hydrolases may cleave the compound and reactivate the toxin. Several conjugated forms of DON such as DON-3-glucoside and DON sulfates, but also of zearalenone and fumonisins have been identified to date. The prominent resistance QTL *Fhb1* is associated with resistance to fungal spread and the ability to inactivate DON by conversion into the less toxic DON-3-glucoside. This dependency initiated discussions on possible hidden risks when introgressing this QTL into wheat cultivars because a considerable fraction of the mycotoxin content might be just masked as glucoside but not circumvented from production.

Based on published and own data we investigated the effect of FHB resistance breeding in wheat on DON and DON-3-glucoside levels and studied the relationship of disease measures evaluated on the plants or the seeds and toxin contents.

The results show that all wheat lines have the ability to convert DON to DON-3-glucoside, independent from their specific FHB resistance level confirming that detoxification of DON to DON-3-glucoside is not a new trait introduced by recent resistance breeding efforts. Our experiments revealed high correlations of FHB symptoms on wheat heads, DON and DON-3-glucoside contents showing that selecting improved lines based on FHB symptoms or DON reduces simultaneously the DON-3-glucoside contamination. The amount of DON-3-glucoside relative to DON contamination varied between 5% and 30% and was influenced by genotypes and environments. Notably the most FHB resistant lines showed the lowest contamination with DON and with DON-3 glucoside, but relatively more DON was glycosylated (up to 30%) compared to susceptible cultivars. Specific resistance QTL (e.g. *Fhb1*) possibly enhance the speed or rate of DON detoxification.

Taken together, breeding of new cultivars with reduced *Fusarium* disease severity will lead to reduced toxin contamination, for the prevalent toxins such as DON, but also for less abundant mycotoxins and masked mycotoxins.

Keywords

Deoxynivalenol · *Fhb1* · Fusarium head blight · food safety · *Triticum aestivum*

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Inferences on resistance stability by the genetic analysis of aggressiveness in *Fusarium* populations

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Abstract

Fusarium head blight (FHB) is a devastating disease of bread wheat (*Triticum aestivum* L.) and other small-grain cereals worldwide. The expression of quantitative pathogenicity is not only controlled by the pathogen and the host, but also by the environment and their interactions. As quantitative resistance/aggressiveness depends on a large number of genetic components from the host and the pathogen, it does not exhibit the boom and bust cycle characterizing qualitative plant pathogen interactions. However, pathogen populations following quantitative interactions remain aggressive in time despite the efforts to control them. In order to offer durable management strategies, a deeper understanding of the mechanisms underlying quantitative aggressiveness is needed.

Pathogenicity assays were used to evaluate and compare 30 *Fusarium culmorum* isolates using as host four winter cereal species (bread wheat, durum wheat, triticale, rye) in a multienvironmental design during two years and two locations. Furthermore, in order to unmask the molecular components of aggressiveness, candidate gene association mapping was performed using a population of 100 *F. culmorum* isolates.

We observed a quantitative relationship between *F. culmorum* isolates and four plant species used as hosts. The multienvironmental trial revealed the factors isolate and isolate by environment interaction as main drivers explaining the variation in the field. Any significant isolate by crop interaction was identified in our data, suggesting no specialization of individual isolates for any specific crop. The candidate gene association analysis allowed the identification of one out of 17 candidate genes associated with field aggressiveness and explaining 10.3% and 6.1% of the genotypic variance for aggressiveness and deoxynivalenol (DON) production, respectively. We hypothesize that changes in gene regulation confer advantages in the response of *F. culmorum* to multiple stresses, especially to the osmotic and oxidative stresses resulting from the plant defense mechanisms.

Keywords

Association mapping · candidate genes · Fusarium head blight · quantitative pathogenicity

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Anther extrusion, a passive resistance factor against Fusarium head blight, is associated with semi-dwarfing alleles

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Abstract

Resistance to Fusarium head blight (FHB) is quantitative, modulated by polygenes and environmental factors. Active and passive resistance factors modulate FHB resistance. The two most important passive traits are plant height and anther extrusion/retention. The pathogen resides on the soil surface, thus successful infection is more likely for shorter plants. Furthermore, shorter plants are more affected by soil humidity and that again supports infection and disease development. Fusarium head blight infection starts inside the floral cavity. Anthers are very fragile; they potentially offer little resistance to the fungus and constitute a preferred target for initial infection. Hence, plant height and anther retention contribute to initial infection and fungal development. Studies showed that manually removing anthers significantly improved FHB resistance, while manually compressing anthers increased susceptibility.

The semi-dwarfing alleles for reduced height *Rht-D1b* and *Rht-B1b* are extensively used in wheat breeding. *Rht-B1b* and *Rht-D1b* cause the same reduction in plant height but the *Rht-D1b* allele has a significantly greater impact on anther retention than the *Rht-B1b* allele. Reduced plant height and a high proportion of retained anthers increase FHB severity. The stronger negative impact of *Rht-D1b* on FHB can be partially explained by the higher numbers of retained anthers. In light of these results, we suggest that the semi-dwarfing allele *Rht-B1b* should be preferred in wheat breeding over *Rht-D1b*.

Keywords

Anther retention · passive resistance · plant height · *Rht* genes · *Triticum aestivum*

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QTL mapping of Fusarium head blight resistance in three related durum wheat populations

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Abstract

Durum wheat (*Triticum durum* Desf.) is particularly susceptible to Fusarium head blight (FHB) and breeding for resistance is hampered by limited genetic variation within this species. To date, resistant sources are mainly available in a few wild relative tetraploid wheat accessions.

In this study, the effect of the well-known hexaploid wheat (*Triticum aestivum* L.) quantitative trait locus (QTL) *Fhb1* was assessed for the first time in durum wheat. Three F_7-RIL mapping populations of about 100 lines were developed from crosses between the durum wheat experimental line DBC-480, which carries an *Fhb1* introgression from Sumai-3, and the European durum cultivars 'Karur' and 'Durobonus', and the breeding line SZD1029K. The RILs were evaluated in field experiments for FHB resistance in three seasons using spray inoculation and genotyped with SSR as well as genotyping-by-sequencing markers. QTL associated with FHB resistance were identified on chromosome arms 2BL, 3BS, 4AL, 4BS, 5AL and 6AS at which the resistant parent DBC-480 contributed the positive alleles. The QTL on 3BS was detected in all three populations centered at the *Fhb1* interval. The *Rht-B1* locus governing plant height was found to have a strong effect in modulating FHB severity in all populations. The negative effect of the semi-dwarf allele *Rht-B1b* on FHB resistance was compensated by combining with *Fhb1* and additional resistance QTL. The efficient introgression of *Fhb1* represents a significant step forward for enhancing FHB resistance in durum wheat.

Keywords

Fhb1 · resistance breeding· tetraploid wheat · *Triticum durum*

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PRAT N, GUILBERT C, PRAH U, WACHTER E, STEINER B, LANGIN T, ROBERT O, BUERSTMAYR H (2017) QTL mapping of Fusarium head blight resistance in three related durum wheat populations Theor Appl Genet 130: 13-27. DOI: 10.1007/s00122-016-2785-0

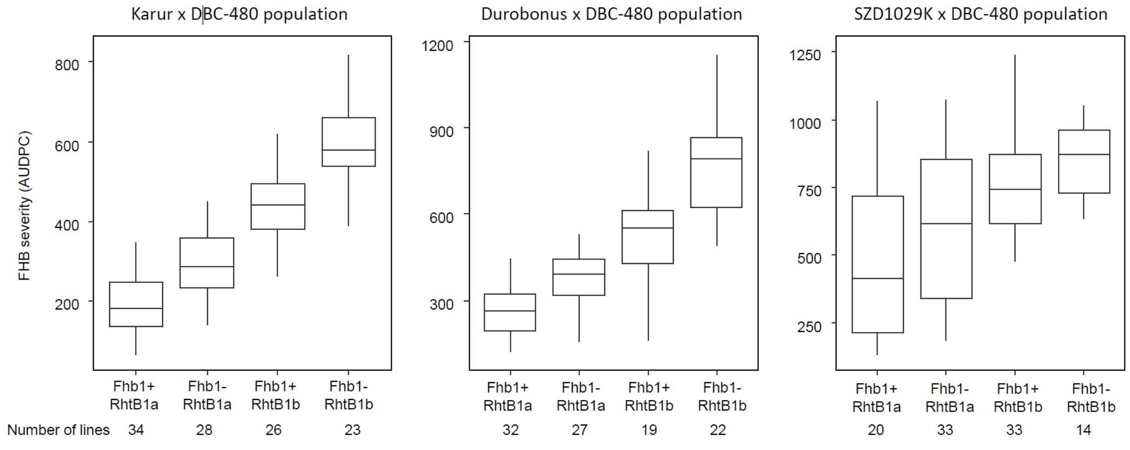

Figure 1: Fusarium head blight (FHB) severity of RILs of three durum wheat populations according to their allele combinations at *Fhb1* and *Rht-B1* loci. Medians are indicated by solid lines; for each subgroup, the number of lines is indicated.

Prat N, Guilbert C, Prah U, Wachter E, Steiner B, Langin T, Robert O, Buerstmayr H (2017) QTL mapping of Fusarium head blight resistance in three related durum wheat populations. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 67. Jahrestagung 2016, 21-23 November, Raumberg-Gumpenstein, p 11. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-45-9

67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2016, HBLFA Raumberg-Gumpenstein, Irdning, Österreich

Recently registered wheat varieties 'Carmina' and 'Ibarra' with improved Fusarium head blight tolerance

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Abstract

Fusarium head blight (FHB) of wheat and the associated mycotoxin contamination is an important problem in some regions of the Czech Republic. To avoid FHB infection and mycotoxin contamination in conventional wheat production the use of tolerant varieties is the best option. Many FHB resistance sources are described in the literature, however, almost all of them have poor agronomic performance compared to adapted varieties. The introgression of 'exotic' FHB resistance QTL into high-yielding elite cultivars often causes the deterioration of important agronomic traits. The recently released winter wheat varieties 'Carmina' and 'Ibarra' were derived from FHB tolerant variety 'Sakura' and exhibit improved FHB tolerance as well as good agronomic traits and can, therefore, recommended in areas of the Czech Republic which are prone to *Fusarium* infections.

Keywords

Fusarium culmorum · *Fusarium poae* · mycotoxin · resistance breeding · *Triticum aestivum*

Introduction

In the Czech Republic, Fusarium head blight (FHB) occurs in winter wheat fields every year. The intensity of infection depends on local climatic conditions, especially temperature and air humidity (CHRPOVA *et al.* 2016); temperatures >6°C together with high humidity, *e.g.* fog and morning dew nearby ponds and rivers, promote FHB infections and mycotoxin accumulation. Minimum or no tillage together with maize as precrop can cause mycotoxin contaminations above the EU maximum limits for food and feed. Areas of the Czech Republic which are especially prone to high levels of FHB infections are displayed in Figure 1.

Material and methods

The recently released winter wheat varieties 'Carmina' and 'Ibarra' were tested for FHB resistance in artificially inoculated field trials during the seasons 2012-2016 at two locations, *i.e.* the Crop Research Institute, Prague, and the Selgen breeding station Stupice. Inoculations were carried out using a spore suspension inoculum of *Fusarium culmorum* and *F. poae* individually at flowering time. 'Carmina' and 'Ibarra' were compared with the following check varieties: 'Arina' (MR, medium resistant), 'Vanessa' (MS, medium susceptible) and 'Biscay' (S, susceptible). FHB symptoms were evaluated 2, 3 and 4 weeks after infection on a 1 to 9 scale (9 = resistant). Infected spikes were harvested for the analysis of the mycotoxin DON (deoxynivalenol) by ELISA.

Resistances to other diseases were evaluated in small plots after artificial infections: leaf rust (Lr), yellow rust (Yr), stem rust (Sr), powdery mildew (Pm) and tan spot (primarily *Septoria*) were evaluated on a 1 to 9 scale (9 = resistant). Three types of artificial frost

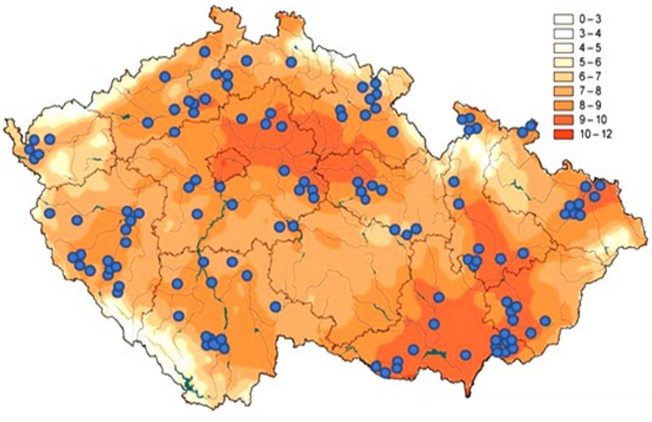

Figure 1: Czech areas with FHB infected wheat samples (blue dots) which exceeded the maximum limit for mycotoxin contamination (2004-2015). Yellow to red colour shades represent the mean annual temperature in the period 1960-1990

Veskrna O, Chrpova J, Sedlacek T, Matyk J, Pospisilova V, Jezek S, Horcicka P (2017) Recently registered wheat varieties 'Carmina' and 'Ibarra' with improved Fusarium head blight tolerance. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed.), 67. Jahrestagung 2016, 21-23 November, Raumberg-Gumpenstein, pp 13-15. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-45-9

resistant tests were done according to methods described by HORCICKA *et al*. (2005).

Results and discussion

Varieties 'Carmina' and 'Ibarra' showed repeatedly low visible FHB symptoms and DON accumulation after artificial *Fusarium* infection. Mean values of the whole testset were 6.1 and 45 mg kg^{-1} for FHB symptom score and DON content, respectively. 'Carmina' was scored as medium tolerant (6.9); DON content was 37 mg kg^{-1}. 'Ibarra' showed also medium resistance (6.7) and it's DON content was only 11 mg kg^{-1} which was lower than the DON content of MR variety 'Arina' (17 ppm). Results from five years are presented in Figure 2 together with two other MR varieties, 'Julie' and 'Viki'.

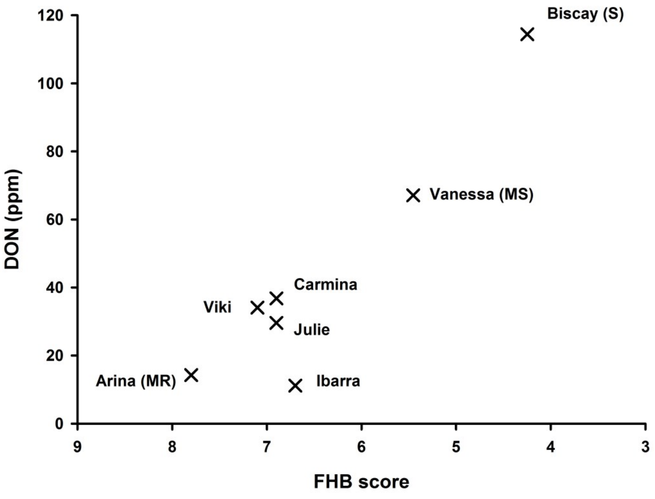

Figure 2: Fusarium head blight (FHB) symptom scores (1 to 9; 9 = resistant) and deoxynivalenol (DON) contamination (mg kg^{-1}) after artificial inoculation with *Fusarium culmorum* and *F. poae* (2012-2016). MR, medium resistant; MS, medium susceptible; S, susceptible

'Carmina' and 'Ibarra' were derived from crosses between 'Akteur' and FHB tolerant 'Sakura'. The good resistance level of the latter variety was already published by REHOROVA *et al*. (2008), CHRPOVA *et al*. (2009) and SIP *et al*. (2010).

Disease resistances and frost tolerance of 'Carmina' and 'Ibarra' are presented in Figure 3. Resistance to yellow rust was at a fairly good level, however, decreased during the epidemics of 2013 to 2015 (appearance of the Warrior and Warrior (-) races) to 4.7 and 5.1 for 'Ibarra' and 'Carmina', respectively. In combination with fungicide treatment, the resistance of these varieties appears to be sufficient for the protection of the plants. Resistances to other diseases, *e.g*. leaf rust, stem rust, tan spot and powdery mildew are at a good level. An advantage of both varieties is their very high frost resistance. Results from artificial frost tests were confirmed by a very hard winter season in 2011-2012.

The high FHB tolerance of 'Carmina' and 'Ibarra' was not impaired by low baking quality. Quality parameters are shown in Table 1. The two varieties were also registered in Slovakia and were classified in the top baking quality classes, i.e. E for 'Ibarra' and AE for 'Carmina'. The varieties have a very high test weight, stable falling number, high protein content and high sedimentation value. Table 1 shows also the quality traits for 'Julie' and 'Viki' which have both E class quality. 'Viki' was registered in Slovakia in 2015 and is in the 3rd year of VCU trials in the Czech Republic and Germany. The FHB resistance of 'Viki' was meanwhile also confirmed by the German VCU trials in which it was one of the most tolerant genotypes to FHB.

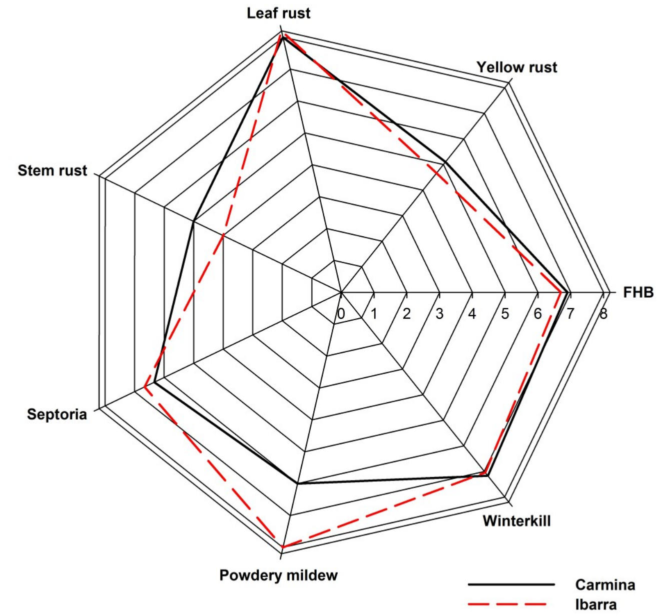

Figure 3: Radar plot of scores of leaf and ear diseases and winterhardness for winter wheat varieties 'Carmina' and 'Ibarra'. Scores are on a 1 to 9 scale with 9 representing the desirable performance

Table 1: Mean performance of Czech winter wheat in main quality traits (2012-2016)

Variety	BQC[1]	PROT	SEDI	SDS	HLW
Carmina	AE	13.2	48	65	815
Ibarra	E	13.1	47	75	820
Viki	E	13.3	47	80	820
Julie	E	13.0	46	71	805
Vanessa	CK	11.5	25	35	760

Variety	BQC	HFN	GLUT	GLX	TGW
Carmina	AE	338	29	86	48
Ibarra	E	368	29	93	46
Viki	E	351	32	93	49
Julie	E	313	28	91	56
Vanessa	CK	273	23	100	50

[1] BQC, baking quality class; PROT, crude protein content (%); SEDI, Zeleny sedimentation value (mL); SDS, SDS sedimentation value (mL); HLW, hectolitre/test weight (kg hL-1; HFN, Hagberg falling number (s); GLUT, wet gluten content (%); GLX, gluten index; TGW, thousand grain weight (g)

Acknowledgements

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2016, HBLFA Raumberg-Gumpenstein, Irdning, Österreich

The leaf rust resistance gene *Lr22a* does not alter other disease resistances, yield or bread making quality

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Abstract

Worldwide, leaf rust is one of the most important disease on wheat. Leaf rust epidemic occur every year in Switzerland, leading to yield reduction. Some leaf rust resistance gene are linked with, more or less, unwanted side effect. For example, *Lr9* could lead to yield reduction, *Lr34* cause a leaf type necrosis and *Lr19* is linked with yellow flour. The efficacy and unintended effects of new resistance genes must be examined prior to utilisation the latter in a breeding programme. In the case of wheat, it is necessary to verify that the resistance gene is still effective and has no negative side effect on other disease resistances, yield or baking quality. The *Lr22a* gene confers resistance to leaf rust at the adult stage (adult-plant resistance), and microsatellite markers linked to it have been identified. To date, this gene is relatively rarely used, and is still effective under Swiss conditions.

Lr22a was introgressed from the spring wheat cultivar 'AC Minto' by 6 backcrosses (BCs) into the susceptible spring wheat cultivars 'CH Campala' and 'CH Rubli'. The presence of *Lr22a* was examined at each BC step using the microsatellite markers *wmc503* and *gwm261*.

The BC lines CH Campala-6BC and CH Rubli-6BC were compared with their original cultivar in multi-location yield trials for four (2012 to 2015) and two (2012 to 2013) years respectively. Simultaneously, resistance to stripe rust (*Puccinia striiformis*), powdery mildew (*Blumeria graminis*), leaf and glume blotch (*Phaeosphaeria nodorum*), Septoria leaf blotch (*Mycosphaerella graminicola*) and Fusarium head blight (*Fusarium graminearum*) were tested using artificial infections. Protein content and Zeleny sedimentation index were determined for each location. The seed harvested from all locations was used for dough- and bread making-quality tests (farinograph, extensograph and Rapid-Mix-Test). The similarity of the resulting two backcross lines (BC lines) CH Campala-6BC and CH Rubli-6BC with their recipient cultivars was verified using a 15K SNP Array.

The number of polymorph SNPs between the BC lines and their their recipient cultivars was low comparing with the number of polymorph SNPs between the two cultivars. As expected, the original cultivars and their essentially derived lines containing *Lr22a* (BC lines) were very similar in heading time, plant height and for morphological traits. Overall, the BC lines showed significantly improved resistance to leaf rust in all trials. In years and at locations with strong leaf rust pressure, we measured a significant yield reduction (up to 13.7%) in the original varieties compared with the improved BC lines.

Resistance to other diseases was very similar (less than 0.5 point difference). In one trial, few stem rust (*Puccinia graminis*) symptoms on the backcross line CH Campala-6BC have been observed. No significant (or very small) differences were observed for protein content, Zeleny index, and rheological or baking parameters. In conclusion, for both genotypes tested, *Lr22a* gene is effective against leaf rust, with no associated negative effects. Nevertheless, we recommend associating this gene with one or more other effective leaf rust resistance genes in order to ensure its durability.

Keywords

Adult plant resistance · marker assisted selection · *Triticum aestivum*

Table 1: Number of polymorph SNPs in a 15K SNP Array

Line/cultivar comparison	Polymorph SNPs
CH Rubli *vs* CH Rubli-6BC	234
CH Campala *vs* CH Campala-6BC	106
CH Campala *vs* CH Rubli	4145

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Table 2: Mean performance values of BC-lines compared to their original cultivar mean values

Trait	BC-Lines	CH Campala, CH Rubli	*P*-value
Grain yield (dt ha^{-1})	55.3	54.7	0.530
Thousand kernel weight (g)	41.8	41.6	0.715
Specific weight (kg hL^{-1})	80.8	80.4	0.158
Heading date (days from Jan 1st)	162.4	162.4	0.861
Plant height (cm)	87.6	88.1	0.541
Lodging (1-9)	1.4	1.4	0.178
Mildew (1-9)	4.3	3.9	0.028
Stripe rust (1-9)	2.5	2.8	0.073
Leaf rust (1-9)	1.4	5.1	0.048
Stem rust (1-9)	2.7	2.0	
Septoria leaf blotch (1-9)	4.1	4.2	0.926
Fusarium (1-9)	3.4	3.6	0.081
Falling Number (s)	340	343	0.849
Protein content (%)	14.2	13.9	0.034
Zeleny (mL)	59.4	59.3	0.860
Wet gluten (%)	30.0	26.7	0.268
Farinograph, water absorption (%)	60.4	59.0	0.319
Farinograph, stability (min)	5.2	4.9	0.273
Farinograph, degree of softening (FU)	107.3	98.5	0.116
Rapid-Mix-Test, volume (mL)	504.0	503.0	0.934
Bread making test, box (mL)	500.5	497.0	0.955
Extensograph, resistance at 5 cm (EU)	201.5	197.0	0.464
Extensograph, extensibility (mm)	370.8	424.3	0.173

67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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Stability patterns of resistance against leaf and glume blotch in wheat

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Abstract

The ascomycete *Phaeosphaeria nodorum* can provoke sever infections on both leaves and glumes of wheat. Leaf blotch can cause the reduction of yield, while infections of the glume can alter the conformation of the developing grain and reduce its quality for baking and as a seed. The outcome of the infection depends characteristically on the age of the plant. Susceptibility increases with plant age. In modern agriculture, the control of the disease relies on measures to prevent the disease (crop rotation, use of certified seeds), the treatment with chemical pesticides and the use of resistant varieties. Resistance against the two diseases is independent one from the other and mostly quantitative and highly conditioned by the environment. For the appreciation of varietal resistance, stability of resistance in different environments and years is of utmost interest. In the present study we test a procedure allowing to screen the resistance in the field of a large number of wheat genotypes with artificial infections taking into account the degree of genotype by environment interactions (G×E) and the earliness of the entries.

For the tests, *P. nodorum* isolates from symptomatic plants are used. Mass production of conidiospores is done on autoclaved wheat grains in Erlenmeyer flasks at 3°C. In the field tests, all entries are infected several times, usually at the start of heading, at the end of heading, at the start of flowering and at the end of flowering. Heading date and at least three scores for each disease are recorded. Disease scoring is done on a 1 to 9 scheme with 1 = no symptom and 9 = the plant organ is completely diseased. Disease severity is calculated by integrating the time of exposure with the severiy scores (AUDPC, area under disease progress curve). Data are corrected with earliness if the distribution of the earliness in the entry population is normally distributed and significant. For this, the population is divided into three homogeneous groups of early, mean and late varieties. A correction coefficient for the late and early group is calculated and added or substracted to the AUDPC value of each variety. Single AUDPC values are then transformed in indices with the average AUDPC value = 100. Here, we report on a seven years experiment at two experimental sites on a set of 18 modern wheat varieties.

By comparing the stability of the index scores, four stability patterns have been identified: (i) stable for both diseases, (ii) unstable for both diseases, (iii) stable for glume blotch, but not for leaf blotch and (iv) stable for leaf blotch but not for glume blotch. Our results indicate that at least four years by environment observations are needed to arrange a variety into one of the stability classes. These results confirm that not only the disease resistances mechanisms of leaf and glume blotches but also their stability over different environments are inherited independently. Stability of resistance sorts out as a major issue for breeding since also genetically close varieties may display distinct resistance and stability patterns. Earliness influences the susceptibility, yet it is not genetically linked to resistance. The correction of the disease scores (*i.e.* indices) according to earliness, allows to normalize the results over serveral years and makes it easier to arrange the genotypes into stability classes. The environmental factors determining the variations have not been investigated here. Yet, G×E and genotype by management (herbicide, fertilisation) interactions (G×M) must be considered in future research.

Keywords

Earliness · genotype by environment interaction · *Phaeosphaeria nodorum* · resistance breeding · *Triticum aestivum* · variety trials

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2016, HBLFA Raumberg-Gumpenstein, Irdning, Österreich

Occurrence of Microdochium leaf blotch in triticale, winter wheat and winter durum in Austria

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Abstract

The leaves of all cereal species as well as many other grasses can be infected by the fungus *Microdochium* sp. (a teleomorph of *Monographella* sp.). Humid weather conditions are essential for the spread of the disease. The symptoms become visible during the late stages of stem elongation and can appear up until the development of the grains. Shriveled grains and serious yield losses can result from infection. The present work is based on observations of winter triticale, winter wheat and winter durum made during VCU (value for cultivation and use) tests in Austria conducted from 2010 to 2016. Estimations of disease severity and disease incidence were made by visual ratings. Compared to the differences observed among cereals affected by mildew and rust diseases, the phenotypic differences among cereals infected by *Microdochium* sp. were less pronounced. A selected set of winter wheat cultivars was described as being moderately, highly or very highly susceptible. Currently, the disease is rarely noticed by European breeders. During the variety registration process, Microdochium leaf blotch is currently not considered to be relevant in a strict sense. One reason for this is that the disease occurs in mixed infections which makes the exact determination more difficult. In Austria, only one fungicide is recognized to control Microdochium leaf blotch.

Keywords

Fungicide · leaf disease · snow mould · *Triticum aestivum* · *Triticum durum* · × *Triticosecale*

Introduction

The ascomycetes *Microdochium nivale* (Fr.) Samuels & Hallett and *Microdochium majus* (Wollenw.) Glynn & S.G. Edwards frequently occur together (KOLEV 2003, HESS 2014) and can affect the plants during various growth stages and in different organs. A long period of snow cover causes damage to winter cereals. Later, infections of the base of the stem, leaves (Figure 1) and ears are possible. The following text describes the leaf infection of triticale, winter wheat and winter durum. The risk of necrosis caused by *Microdochium* sp. has been steadily increasing in the grain production of cereals (HESS 2016). Because the disease rarely occurs alone, but rather in a mixture of infections, it is nearly impossible to quantify the damage.

Figure 1: Symptoms of infections with *Microdochium* sp. on leaves of winter wheat

Material and methods

Data collected during the official VCU trials and other field trials conducted with triticale (2013, 2016), winter wheat (2012, 2013, 2015, 2016) and winter durum (2010, 2013) were evaluated. The trials were conducted in the production areas located in the north-eastern plains and hills, alpine foothills, eastern alpine foothills, south-eastern plains and hills and the Carinthian basin in Austria. The results of these trials are available for the following sites: Großnondorf (Gro, GrF, Hollabrunn district), Gerhaus (Ger, Bruck an der Leitha), Pultendorf (Pul, St. Pölten), St. Florian (Flo, Linz-Land), Ritzlhof (Rit, Linz-Land), Marchtrenk (Mar, Wels-Land), Reichersberg (Rei, Ried im Innkreis), Warth (Wat, Neunkirchen), Gleisdorf (Gle, Weiz), Eltendorf (Elt, Jennersdorf) and St. Paul (StP, Wolfsberg). During these trials, one or two factors were assessed and 3 to 4 replicates were carried out on plot sizes that ranged from 8.1 to 12.8 m^2. The symptoms of *Microdochium* sp. infection were assessed by visually rating the disease severity and disease incidence using a scale of 1 to 9 (1 = no infestation, 9 = very strong infestation). The flag leaf (F) and the two leaves below the flag leaf (F-1, F-2) were examined.

Results and discussion

Microdochium leaf blotch has been observed more frequently in recent years, occurring after rainy periods and prolonged periods during which leaves remained wet. The life cycle of the disease is dependent upon the presence of saprophytic mycelium in infected straw. During the growing season, conidia are spread by splash dispersal, and the ascospores, which are formed in perithecia, are spread by wind dispersal (OBST & GEHRING 2002). Characteristic symptoms are greyish green and later brown-grey oval lesions on the initially water-soaked leaf blades or leaf sheaths (Figure 1). During the spring and early summer of 2009, such symptoms were significantly evident on wheat and triticale growing in the alpine

Oberforster M, Flamm C (2017) Occurrence of Microdochium leaf blotch in triticale, winter wheat and winter durum in Austria. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed.), 67. Jahrestagung 2016, 21-23 November, Raumberg-Gumpenstein, pp 21-22. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-45-9

foothills region (HUSS 2010). Subsequently, the disease was occasionally determined during field trials. Winter durum was very highly affected at the location Großnondorf in 2010. In this region, rain fell on 33 days from the beginning of May until the middle of June. In 2016, Microdochium leaf blotch symptoms were often observed in Austria on triticale, rye, common wheat and durum wheat plants. A wet weather period that had lasted several weeks preceded these observations. In Germany in 2013 and 2016, the disease damaged cereals in many regions. Because Microdochium leaf blotch often co-occurs with Septoria leaf blotch (caused by *Septoria nodorum* or *S. tritici*), tan spot (*Drechslera tritici-repentis*), yellow rust (*Puccinia striiformis*), leaf rust (*P. triticina*), leaf scald (*Rhynchosporium secalis*) or non-parasitic leaf blotches, significant difficulties in determining the infestation are encountered.

The severity and incidence of the leaf infestation varies according to the environmental conditions, the growth stage of the plants and the resistance of the individual genotypes (Figure 2). In winter wheat, the majority of the current cultivars seem to be moderately to highly susceptible to Microdochium leaf blotch (Table 1). A fungicide (1.5 L ha^{-1} Adexar®) that was applied during the late stages of stem elongation up until the formation of the seed heads, which is effective against brown rust, yellow rust and Septoria leaf blotch, also reduced the symptoms of *Microdochium* sp. infection. On average, the fungicide treatment reduced the Microdochium leaf blotch score by one unit (Table 2). However, the fungicidal effect was significantly weaker than that observed in rust diseases. This may have been in part due to the application date, which may have been too late.

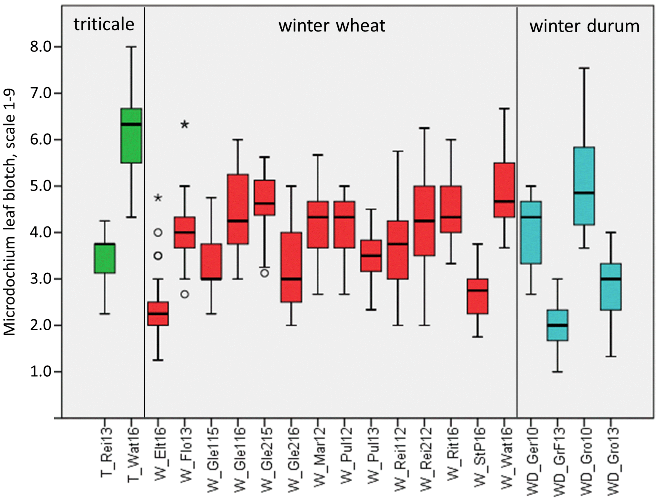

Figure 2: Infestations of triticale, winter wheat and winter durum with Microdochium leaf blotch (20 trials; 2010-2016; 12-30 cultivars and breeding lines; 1-9 score scale)

Table 1: Susceptibility of selected winter wheat cultivars for Microdochium leaf blotch on the basis of observations made between 2012 and 2016

moderate	moderate – high	high	high – very high
Chevalier	Beryll, Findus,	Advokat	Arktis
Florencia	Frisky, Gideon,	Angelus	Johnny
Pankratz	Henrik, Lukullus,	Hewitt	Papageno
Spontan	Mulan, Norenos,	Kerubino	
	Pedro, Richard,	Sailor	
	Sax, Siegfried		

Table 2: Effects of fungicide application (1.5 L ha^{-1} Adexar®, 62.5 g L^{-1} active substances epoxiconazole and fluxapyroxad, respectively) on the occurrence of Microdochium leaf blotch in winter wheat (7 trials; 2012-2016; 3-30 cultivars and breeding lines; 1-9 score scale)

Site × Year	Pul 2012	Pul1 2013	Pul2 2013	Gle 2015	Wat 2016	Gle 2016	StP 2016
n[1]	4	4	30	3	3	3	3
Ctrl	4,5	3,5	3,9	4,1	5,3	4,3	2,3
Treat	3,5	3,1	3,2	2,8	4,2	2,6	1,6
Diff	-1,0	-0,4	-0,7	-1,3	-1,1	-1,6	-0,8

[1] *n*, number of cultivars; Ctrl, no fungicide treatment; Treat, fungicide treatment; Diff, difference in Microdochium leaf blotch score between control and fungicide treatment

According to studies conducted in Germany, the active substances prochloraz, epoxiconazole, prothioconazole, tebuconazole and combinations of azoles and carboxamides inhibit the growth of these fungi. Strobilurins were shown to be only weakly effective. Because the fungicides have no curative effect on established infections, early applications of fungicides are necessary. In field trials conducted with winter wheat, yield gains between 7 and 25 dt ha^{-1} were achieved (HANHART 2014, 2017). In Austria, only the plant protection product Zantara®, which contains the active substances tebuconazole and bixafen, has been authorized for use against Microdochium leaf blotch. It is possible to spray barley, rye, triticale, common wheat and durum wheat from the period of stem elongation up until the end of flowering (BBCH 31-69).

Within the framework of the official variety testing, winter barley, rye, triticale and winter oats were assessed for their susceptibility to the soil-borne pathogen snow mould (*Microdochium* sp.). The Microdochium leaf blotches visible between BBCH 37 (flag leaf still rolled) and BBCH 83 (early dough stage) are currently not considered to be relevant in a strict sense. The symptoms are only occasionally detected. Due to the increased importance of Microdochium leaf blotch during recent years in Austria, the integration of the symptomatic characteristics of this disease would be justified as an additional criterion of the VCU tests.

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21.-23. November 2016, HBLFA Raumberg-Gumpenstein, Irdning, Österreich

Interaction between yield, quality and occurrence of diseases in winter wheat grown after different pre-crops

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Abstract

Economy is forcing farmers into a reduced number of crops in crop rotation but interaction between wheat and unfavourable pre-crops can cause yield losses. The reaction of eight winter wheat cultivars grown in the Czech Republic to four different pre-crops (OSR or pea, grain maize, second wheat, monoculture wheat) was evaluated. Testing focused on grain yield, quality and occurrence of diseases in the field. Yield losses were confirmed for unfavorable pre-crops.

Keywords

Crop rotation · grain yield loss · *Pisum sativum* · *Triticum aestivum* · *Zea mays*

Introduction

The pre-crop can significantly affect growth, yield formation and quality of common wheat (*Triticum aestivum* L.). In dry seasons the amount of water removed from soils by preceding crops can have a major effect on the growth of following crops. In annual cropping cycles, the inclusion of deep-rooting rotation crops removes significantly more water which may result in significant yield loss in the subsequent wheat crop is some seasons.

Material and methods

The reaction of eight winter wheat cultivars (Table 1) grown in the Czech Republic after four different pre-crops (oilseed rape or pea, grain maize, second wheat, monoculture wheat) was evaluated in field trials from 2014 to 2016. Grain yield, quality and occurrence of diseases were recorded in the field trials.

Results and discussion

Significant differences in the reaction of the tested cultivars were observed in the years 2014 to 2016. Heavier infections with Fusarium spp. and eyespot was observed after corn and wheat monoculture, respectively. Varieties which are suitable for planting after unfavorable pre-crops were identified, e.g. the highest level of tolerance to planting after corn was detected in ‘Turandot’; ‘Penelope’ and ‘Vanessa’ turned out to be suitable for monoculture wheat; ‘Turandot’ and ‘Annie’ were less infected by eyespot; most susceptible to Fusarium spp. were ‘Vanessa’ and ‘Hermann’; ‘Turandot’ and ‘Vanessa’ were the overall highest yielding cultivars across all pre-crops (Table 2).

Pea (*Pisum sativum*) is a good pre-crop and might boost yield of the following crop considerably. Interactions between wheat and break crops are complex because there are variable roles of N, water, disease and weather. A good pre-crop for wheat can im-

Table 1: Description of the investigated wheat material (BQG, baking quality group: E, elite - most suitable for baking; A, high-quality; B, additional - suitable for use in mixtures; C , other use - unsuitable for baking)

Cultivar	Year of release	BQG	Earliness	Winter hardiness
ANNIE	2014	E	mid-early	good
JULIE	2014	E	early	very good
ELLY	2010	A	early	good
BOHEMIA	2007	A	early	very good
TURANDOT	2012	A	mid-early	medium
PENELOPE	2015	A	mid-early	good
VANESSA	2013	C	mid-early	medium
HERMANN	2004	C	mid-late	mid-low

Bížová I, Palicová J, Hanzalová A, Bláha T (2017) Interaction between yield, quality and occurrence of diseases in winter wheat grown after different pre-crops. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed.), 67. Jahrestagung 2016, 21-23 November, Raumberg-Gumpenstein, pp 23-24. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-45-9

pact the economics of the farming system. Pre-crops such as pea can provide residual nitrogen to following wheat crop. In addition, a disease break may result in a healthier wheat root system, enabling the crop to use soil N and applied N fertilizer more efficiently and thereby realize higher yields.

For corn (*Zea mays*) the importance of disease resistance was proved in crop rotations. A lot of varieties had the same yield or higher then in pre-crop plant system supposed to be more favorable. Especially important is the resistance to Fusarium head blight (FHB) and non accumulation of mycotoxins. Relatively higher resistance to FHB has variety 'Turandot' variety which was found to be medium resistant. 'Turandot' shows less FHB symptoms than other varieties.

If wheat is grown after wheat (second wheat) or even in monoculture foot rot diseases become prevalent. Therefore, it's important that the wheat variety carries effective resistance against *e.g.* eyespot (*Pch1*). The natural occurrence of strawbreaker disease complex was assessed in a parallel field trial. A lower infection by eyespot (*Pseudocercosporella herpotrichoides*; teleomorph *Tapesia yallundae*) was proved in cultivars with the *Pch1* gene in both trials. We can conclude that *Pch1* confirms satisfactory control of eyespot in field conditions with natural infection pressure and moreover in artificial infected plots too. Seed treatment seems to be more important in cultivars without *Pch1*. Seed treatment can significantly decrease the infection by eyespot and consequently yield losses.

With respect to quality traits 'Annie' exhibited the highest values for crude protein content and specific (hectolitre) weight across all pre-crops. Significantly lower protein contents were observed in wheat grown after wheat (on average -1,3%), whereas a positive pre-crop effect can be seen for pea. Specific weight was the most stable quality trait across all pre-crops. Differences in Zeleny test between planting after pea and maize were highest for 'Julie' and 'Penelope' with -4 and -3 mL, respectively (Table 3). Effects of pre-crops on quality traits could be observed in all quality groups.

Table 2: Grain yield (t ha^{-1}) of winter wheat after different pre-crops (2014-2016). Highest values are printed in bold.

Cultivar	2014				2015				2016			
	pea	maize	wheat	mono[1]	pea	maize	wheat	mono	pea	maize	wheat	mono
ANNIE	11.3	11.1	7.9	8.1	12.4	11.4	8.2	7.2	12.5	12.3	8.4	7.1
JULIE	**12.8**	11.4	8.3	8.0	12.5	12.3	8.1	7.5	13.2	**13.0**	8.5	8.1
ELLY	12.3	11.2	7.9	7.3	12.0	11.2	8.4	7.1	13.4	12.9	8.0	7.9
BOHEMIA	10.4	10.2	7.6	7.1	11.8	11.6	8.9	7.2	12.9	**13.0**	9.0	7.4
TURANDOT	12.5	**12.6**	8.6	8.1	**14.0**	13.4	8.6	8.7	12.9	12.7	10.0	8.0
PENELOPE	11.4	11.1	9.0	**8.6**	13.4	12.8	9.0	8.0	12.6	12.7	8.3	7.1
VANESSA	11.6	11.0	**9.1**	8.1	13.7	**13.5**	**9.5**	**8.8**	**13.7**	12.8	**10.2**	8.3
HERMANN	10.4	9.7	8.5	7.9	13.1	12.1	9.2	8.3	13.3	12.3	10.1	**8.5**

[1] mono, wheat monoculture

Table 3: Quality traits of winter wheat after different pre-crops (2014-2016). Highest values are printed in bold.

QG[1]	Cultivar	Pea				Corn				Wheat monoculture			Wheat		
		PROT	HLW	HFN	SEDI	PROT	HLW	HFN	SEDI	PROT	HLW	HFN	PROT	HLW	HFN
E	ANNIE	**14.4**	**85.0**	432	65	**13.4**	**81.1**	396	65	**13.1**	**84.0**	448	**12.4**	**84.5**	456
E	JULIE	13.4	83.3	422	**70**	13.0	79.7	**419**	**66**	12.6	81.2	379	11.2	80.7	445
A	ELLY	13.8	84.4	**488**	52	13.3	80.0	399	53	13.2	82.7	362	11.6	82.8	**474**
A	BOHEMIA	13.6	80.7	441	63	13.0	78.7	407	60	13.0	78.8	432	11.5	79.3	461
A	TURANDOT	13.0	82.4	356	54	13.1	78.8	346	53	12.3	81.9	316	11.3	81.8	345
A	PENELOPE	12.8	82.8	437	64	12.7	79.1	396	61	12.3	81.4	**451**	11.1	81.2	439
C	VANESSA	11.8	79.6	351	27	11.7	74.6	344	27	11.3	78.9	422	10.8	79.0	362
C	HERMANN	12.2	77.4	344	23	12.6	75.4	366	24	11.0	79.1	373	10.6	78.3	424

[1] QG, baking quality group; PROT, crude protein content (%); HLW, hectolitre (specific) weight (kg hL^{-1}); HFN, Hagberg falling number (s); SEDI, Zeleny sedimentation volume (mL)

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Virus diseases in grain legumes - Situation in Austria 2016

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Abstract

Typical symptoms of virus infection such as large patches of yellowish and dwarfed plants with poorly developed flowers and pods appeared in 2016 first in pea (*Pisum sativum* L.) at the beginning of June and shortly after in faba bean (*Vicia faba* L.). At mid to late June large acreages of peas and faba beans of Lower and Upper Austria were affected. At the beginning of July early infected faba beans showed already pre-mature ripening and lost leaves. Plants which were infected later showed typical chlorosis. The level of infection and yield losses were different, reaching in some cases complete crop failures. In green peas, losses between 35 and 100% were recorded in Lower Austria. Crop yields of 1000 kg ha^{-1} instead of 5000 kg ha^{-1} for faba beans were reported for the Tullnerfeld region. Typical symptoms of stunted growth, chlorosis and significantly reduced pods were also found in lentils (*Lens culinaris* Medik.) and in vetch (*Vicia sativa* L.). Because of the great incidence of virus infection a monitoring was establihed by the Austrian Agency for Health and Food Safety (AGES) at the beginning of June. The analysed samples were mostly faba beans, but also peas, lentils, vetch and chick peas (*Cicer arietinum* L.) from Upper and Lower Austria, Styria and Burgenland. The samples were screened for different viruses using RT-PCR.

Pea necrotic yellow dwarf virus (PNYDV), a Nanovirus was confirmed in 76% of the investigated legume samples. *Pea enation mosaic virus* (PEMV) was detected in 45% of the samples. A few samples were positiv for Potyviruses (*Bean yellow mosaic virus*, BYMV, and *Pea seed borne mosaic virus*, PSbMV) and Poleroviruses. *Bean leaf roll virus* (BLRV), *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV) were not detected in the investigated samples. In November 2016 PNYDV was confirmed in various green manure legumes, *i.e.* faba beans, vetch, pea and grass pea (*Lathyrus sativus* L.).

PNYDV was identified and described for the first time in green peas in Germany in 2009, and was first detected 2010 in Austria in green peas and in faba beans. In 2016 a countrywide outbreak on faba beans was reported for the first time in Germany, and also lentil and vetch were described for the first time as natural hosts in Austria and Germany. Up to 2009, Nanoviruses were only known in warmer regions of the world like North Africa, Asia, the Middle East and Australia causing problems in legume crops. The name Nanovirus refers to the small size of the virions and the observation that this viruses are causing dwarfing in their hosts.

Figure 1: Symptoms of *Pea necrotic yellow dwarf virus* (PNYDV) in faba bean: Stunted chlorotic and already leafless plants

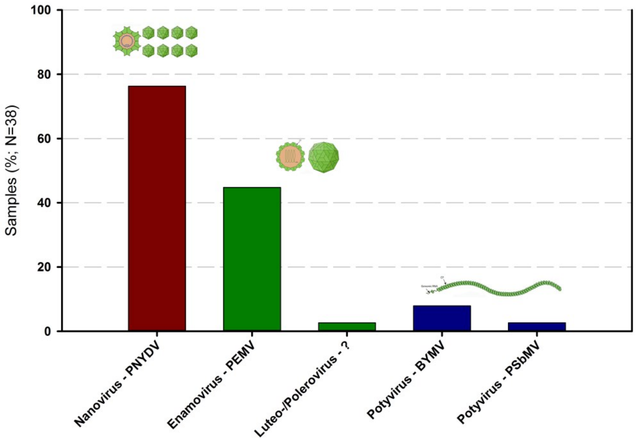

Figure 2: Viruses detected 2016 in Austrian samples of chickpeas, faba beans, field peas, lentils and vetch

Nanoviruses are transmitted by aphids in a non-propagative circulative manner and are restricted to the phloem. As other phloem limited viruses, Nanoviruses are not known to be transmitted by seed or by mechanical inoculation. The host range of Nanoviruses is mainly limited to *Fabaceae*.

Grausgruber-Groeger S, Huss H (2017) Virus diseases in grain legumes - Situation in Austria 2016. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 67. Jahrestagung 2016, 21-23 November, Raumberg-Gumpenstein, pp 25-26. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-45-9

Artificial inoculations at the Julius Kühn Institute (JKI), Braunschweig, Germany, revealed that besides peas, field beans, lentils, common vetch and grass pea also crimson clover (*Trifolium incarnatum* L.) or Mediterranean melilot (*Melilotus sulcatus* Desf.) are host plants for PNYDV. Investigating different cultivars of pea and field beans no resistances against PNYDV were detected. Legumes which could not be infected artificially up to know at JKI Braunschweig are for example alfalfa (*Medicago sativa* L.), common bean (*Phaseolus vulgaris* L.), red (*Trifolium pratense* L.), white (*T. repens* L.) and reversed clover (*T. resupinatum* L.), winter vetch, sainfoin (*Onobrychis viciifolia* Scop.) and soybean (*Glycine max* (L.) Merr.).

Nanoviruses are transmittet by aphids, therefore, early sowing, control of aphids and avoiding pulse crops being host plants for PNYDV in green manures are important tools to restrict virus infections. One practical experience with Nanoviruses in warmer regions and viruses appearing in our climate is that serious outbreaks occure only in a distanc of a couple of years. Nevertheless, causing yield losses up to complete crop failures at early infections, PNYDV is a pathogen we have to take into account seriously. PNYDV is a pathogen we only know for a few years and information about the epidemiology is limited. For better control strategies we have to obtain a better understanding of the distribution, natural hosts and vector transmission of PNYDV as well as the interrelationship between epidemic build-up and climatic conditions.

Keywords

Nanovirus · Pea nectrotic yellow dwarf virus · PNYDV · pulses

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Development of *Tilletia caries* during three generations of seed multiplication – Results obtained from project CARIES

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Abstract

In the scope of a research project funded by the Ministry for Agriculture, Forestry, Environment and Water Management, the characterisation of the seed borne infection cycle of *Tilletia caries* was investigated on three winter wheat varieties under Austrian growing conditions. The tested varieties were 'Arnold', 'Antonius' and 'Capo' which were cultivated for a period of three years at two different locations (Fuchsenbigl and Großnondorf). The goal of investigation was the progress of infection level from the first to the third growing season, concerning the factors area of cultivation, sowing date (two dates) and variety.

Twenty-four samples with defined seed infection levels were cultivated under the mentioned conditions in two repetitions. After the first growing season, infected plants on each field plot were counted, followed by the harvest of the samples and seed testing concerning infection rates of *T. caries*. In autumn, the harvested material was cultivated again. This process was repeated the following two years.

The results after three years of testing were significant, allowing conclusions for practice. One clear result is the huge difference regarding the epidemic pressure on the two locations tested. While at Fuchsenbigl the disease development was still moderate after three generations of growing, at Großnondorf the pathogen had developed highly already in the second year. In the third growing season the plants at Großnondorf were more or less completely deteriorated.

The plants grown at the early sowing dates (early to mid October) showed less infection during the three years than plants grown at the late sowing dates (end October to early November). This was observed at both locations.

The varieties showed constant differences in disease development regarding locations and sowing dates. The results are comparable to the characterisation of the varieties in the VCU trials; in Austria the characterisation of susceptibility to *Tilletia* is obligatory for the organic VCU trials. 'Capo' and 'Arnold' showed significantly higher infection than 'Antonius'. Nevertheless, even when differences within the varieties are detectable, the current level of tolerance is not sufficient to control the disease completely, considering those varieties which are currently used in organic farming in Austria.

The results also show a strong correlation between the infection level of seeds grown, the number of infected plants on the field and the infection level of seeds harvested. A multiannual seed propagation process is only possible, if the infection of the basic seed is at the lowest possible level. Seed infection levels below 10 spores per kernel which is the legal maximum limit for untreated seeds according to the Austrian Seed Regulations gave sufficient safety to grow healthy plants. Especially when development conditions are favourable for the pathogen (*i.e*. location, sowing date, susceptibility of variety) more than one generation of seed propagation without treatment (means the use of untreated seeds two or more times) should be considered as risky.

Based on the project results it can be summarised, that the development of *Tilletia caries* depends on numerous environmental and technical factors. Since no highly tolerant varieties are available at a large scale, a comprehensive risk assessment is of high priority for sustainable seed production to avoid disastrous *Tilletia caries* outbreaks.

Keywords

Common bunt · organic seed production · *Triticum aestivum* · winter wheat

Acknowledgements

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Soil-borne infection of common bunt (*Tilletia caries*) – Results of the project CARIES

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Abstract

Reports about soil-borne infection of wheat with common bunt (*Tilletia caries*) have been increasing in recent years in Austria. The soil-borne infection and viability of spores were studied in the project CARIES. In three year field trials in Vienna, plots were contaminated with spores artificially. A bunt susceptible wheat variety was cultivated at three different sowing depths. In an additional treatment the soil surface was recompacted after sowing. Soil-borne infection was proven all three years. The infestation level was influenced by the way of soil contamination but not by sowing depth. When the soil surface was recompacted after sowing the infestation tended to decline. In two year field trials in Großnondorf two winter wheat varieties with different susceptibility to common bunt were cultivated in plots with different spore contamination levels. The results confirmed the risk of soil-borne infection, the spread of bunt spores by soil cultivation, sowing and harvesting and demonstrated the different susceptibility of wheat varieties concerning soil-borne infection. The viability of spores was tested, performing pot trials with three different substrates in a time series from one to eight weeks. The viability of spores was influenced by the substrate and decreased rapidly. Factors like the moisture of substrate (70% or 85% of water holding capacity) and the compaction of substrate did not influence the viability of spores. The viability was additionally tested under field conditions. After eleven weeks remaining in the field a small percentage of spores was still viable and able to infect seed.

Keywords

Disease cycle · disease prevention · organic farming · spore viability · *Triticum aestivum*

Introduction

Common bunt (*Tilletia caries*) has been spreading in Austria and in several European countries in recent years (BORGEN 2000, WEINHAPPEL 2011, VOIT *et al*. 2012a). The number of cases where soil-borne infection was reported has also been increasing. Drawing attention to the soil-borne infection cycle is important as disease prevention strategies and measures need to be adapted especially for organic farming. In a three-year project from 2013 to 2016 the soil-borne infection and the viability of spores were investigated performing field- and pot trials.

Material and methods

Field trials

At Essling, Vienna, plots were artificially contaminated with spores to investigate the soil-borne infection of common bunt (*T. caries*) on a fluvisol in a three-year trial. The testing area was divided into four blocks, *i.e*. the control without spores (a) and three blocks with different contaminations: (b) distribution of spores on the soil surface by watering a spore solution; (c) mix of spores and sieved soil worked into the soil with a wooden rake from 0 to 7 cm depth; (d) combination of ‘b’ and ‘c’ with the double amount of spores. All four blocks got the same amount of water to ensure comparable starting conditions.

The amount of spores applied at blocks ‘b’ and ‘c’ was 2 g m^{-2} and at block ‘d’ 4 g m^{-2}. The spores originated from the region and were harvested in 2013. Winter wheat was sown with a precision seeder with a distance of 3 cm between kernels and six rows per plot at the beginning of October two to five days after spore distribution. Certified seeds of ‘Capo’, a variety susceptible to common bunt (WEINHAPPEL *et al*. 2013), were used. In every block four plots of 5.5 m² in four replications were cultivated in strips. Three variants of seeding depths, *i.e*. 1-2 cm, 2-4 cm and 4-6 cm were tested and one additional variant where the soil was recompacted after sowing. Two strips with 2-4 cm seeding depth were cultivated next to each other and then each partly recompacted with a tractor by driving over the the strips two times. Per plot about one third of the area was recompacted and two thirds were not recompacted. The field design was maintained in all three years. The infested plants were counted per plot. At Großnondorf (Lower Austria) two winter wheat varieties, ‘Capo’ and ‘Antonius’, with different susceptibility to common bunt (WEINHAPPEL *et al*. 2013) were cultivated in plots with different soil contamination levels on a chernozem in a two-year field trial. In both years the soil contamination was achieved by threshing infested wheat plots from a preceding field trial. Within the project 96 plots of 5 m² per year divided in two blocks, ‘A’ and ‘B’, differing in sowing date were threshed. The infestation level of the wheat plots before threshing was in the first year in block A between 0 and 5.6 (median 0.05)

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and in block B between 0 and 7.6 (median 0.1) infested plants per m². In the second year in block A between 0 and 22 (median 0.25) and in block B between 0 and 90 (median 2.3) infested plants perm². Due to the field design the infestation was ascending from one side of the trial to the other with the effect that neighbouring plots were exposed to a more or less similar spore load. Eleven and 13 weeks after threshing certified seeds of 'Antonius' and 'Capo' were sown in strips exactly on the previously contaminated plots. The number of infested plants per plot were counted.

Viability of spores

Spore viability was tested in three different substrates at two moisture contents related to the water holding capacity and in one additional compaction variant. The basic substrate ('soil substrate') consisted of an agricultural soil (a calcareous grey fluvisol) and quartz sand in a 1:1 (w/w) ratio. The further two substrates ('compost substrates 1 and 2') consisted both of the basic substrate with two different composts in a 7:3 (w/w) ratio. Per pot 1 g of bunt spores was distributed homogeneously in the respective substrate. The pot volume was 2.4 L with 16 cm diameter and 12 cm depth. During filling the pots the substrates were compacted with a 2 kg weight to obtain a comparable density. One extra compaction variant was prepared with the soil substrate using a weight of 240 kg also at two moisture contents. All pots were watered with distilled water to 75% and 80% of the water holding capacity (WHC) of the substrates, respectively. Every variant was prepared in four replications, alltogether 32 pots. The water evaporation was determined by weighing each pot and kept at 75% and 80% WHC, respectively, all time. The sampling was carried out four times in a time series after 1, 2, 4 and 8 weeks. The spores were extracted by wet sieving according to BABADOOST & MATHRE (1998). Germination tests of the spores were conducted by producing a spore-water suspension and pipetting it on water agar (WILCOXSON & SAARI 1996). Chloramphenicol was added to the water agar. The samples were incubated 4 days at 20°C in darkness and then changed to 12 h UV-light/darkness for further 12 to 14 days. The number of spores that germinated and produced sporidia were counted under a light microscope.

In an additional experiment 60 bunt balls were buried in the soil remaining there for 3, 7 and 11 weeks between mid July and end September 2015. They were buried in the field in Essling each in a distance of 10 cm and in 10 cm depth. After extracting the bunt balls the viability of spores of 3×5 bunt balls (5 per period) was analysed. The spores of the remaining 45 bunt balls were used to inoculate certified seed of 'Capo' by dry application with about 5000 spores per kernel. The inoculated seed was cultivated in 3 plots of 6 m² with a rate of 500 kernels per m².

Results and discussion

Field trials

Soil-borne infection could be proven in all three years of field trials. Figure 1 shows that the infection level at Essling was lower in the first year with an average of 12% in comparison to the second and third year with about 40%. In every year the differently contaminated plots show a significant lower infestation of winter wheat when the spores were distributed on the soil surface ('b') in comparison to the ones worked into the soil ('c' & 'd'). Sowing depth showed no effect on the infestation level (Figure 2). The deeper sown seed was expected to develop higher infestation rates because the fungi has more chance to infect the seedling in the soil. This hypothesis couldn't be confirmed. In the third year even a significant reverse effect could be observed as the infestation decreased with the sowing depth in all contamination variants.

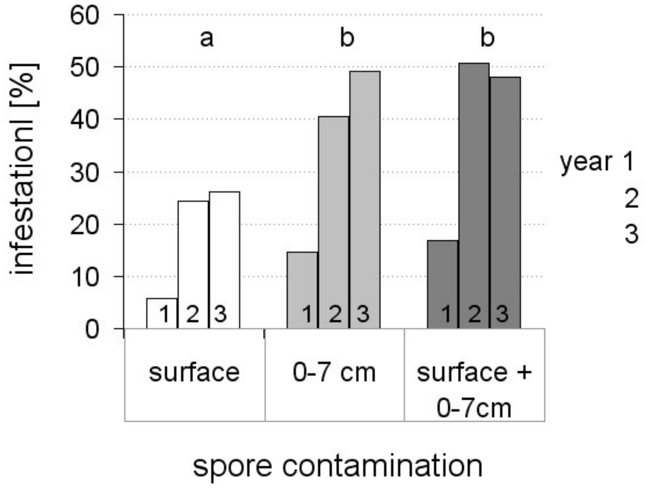

Figure 1: Infestation of winter wheat 'Capo' with common bunt (*Tilletia caries*) by soil-borne infection in a three year field trial in Essling. Artificial soil contamination with spores distributed on soil surface by watering, worked into the soil at a depth of from 0 to 7 cm and by a combination of both inoculation methods. Variants with different letters are significantly different from each other

Considering environmental factors like dry conditions the seedling develops faster in a deeper and, therefore, more humid soil layer. But usually the soil moisture, which favours seed germination, also favours spore germination (MATHRE 2000). BABADOOST *et al*. (2004) reported that the percentage of germination of *T. indica* was significantly higher when spores have been buried at 25 cm than at 2 or 10 cm and he mentioned in this context the influence of different temperatures and moisture in the different soil layers. Cool soil temperatures between 5 and 10°C (VOIT *et al*. 2012b) are the optimum for germination of bunt spores, but temperatures above 20°C result in very low infections.

Figure 3 presents an overview of the recompaction variants. When the soil surface was recompacted after sowing the infestation tended to decline in all three years. A possible explanation is that a slightly compacted soil suports the germination of winter wheat (DEJONG-HUGHES *et al*. 2001) which gives the crop a competitive edge over the fungi. A good vigour and a fast germination are decisive factors for the seedling to escape the risk of infection. The temperature optimum for germination of winter wheat is between 15 and 30°C. A sowing date, which is accompanied by low soil temperatures, will decelerate germination and will make a bunt infection more probable. Our results suggest, however, that an 'early' or 'late' sowing date, as well as the sowing depth can't be taken as simple rules of thumb to avoid infections.

At the field trial in Großnondorf the soil-borne infection of two wheat varieties was evaluated after the soil was contaminated with bunt spores. The way of soil contamination was compareable to practical conditions, *i.e*. spores are deposited on the soil at harvest by threshing infested wheat lots. A further spreading of the spores in the soil takes place by subsequent soil cultivation, by sowing and also by the tillage equipment. In Figure 4 the relation

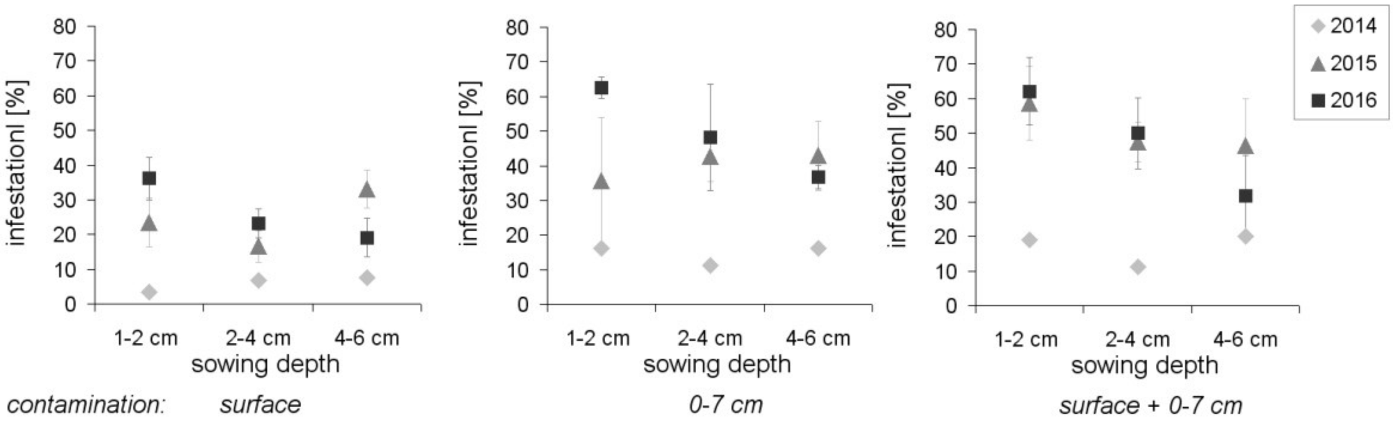

Figure 2: Infestation of winter wheat 'Capo' with common bunt (*Tilletia caries*) by soil-borne infection comparing different sowing depths in a three year field trial in Essling with artificial soil contamination (spores distributed on soil surface by watering, worked into the soil at 0 to 7 cm soil depth and a combination of both methods)

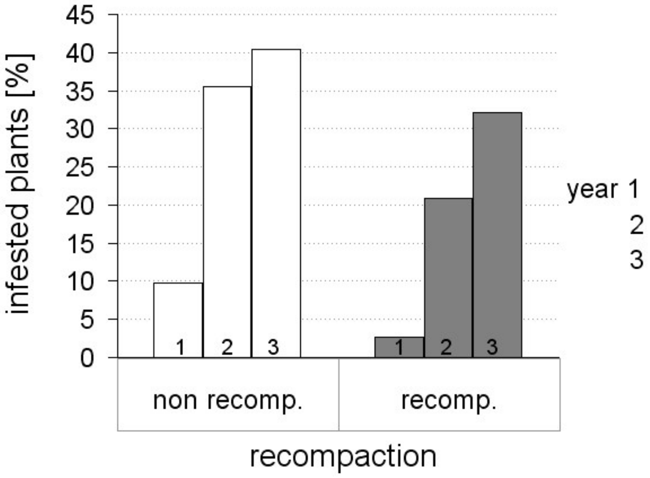

Figure 3: Infestation of winter wheat 'Capo' with common bunt (*Tilletia caries*) by soil-borne infection in a three year field trial in Essling with artificial soil contamination comparing recompacted and not recompacted soil surface

between the initial infestation and the resulting soil-borne infection in the following wheat crop is demonstrated for two years. In the first year between 0 and 2 infested plants per m² were found in the plots of block A and between 0 and 3.4 plants per m² in block B. 'Antonius' and 'Capo' did not differentiate significantly with regard to susceptibility to *Tilletia caries*. In total, the amount of infested plants in the field before threshing was 317 and the resulting infestation by soil-borne infection in the following wheat crop was 257, which corresponds to a factor of approximately 0.8. In the second year the soil-borne infection in block A was between 0 and 18 infested plants per m². The initial infestation in block A correlated with the subsequent soil-borne infection of 'Antonius' with $r = 0.44$ and 'Capo' with $r = 0.55$. In block B the infection was between 0.8 and 66 plants per m². The initial infestation level was much higher with 0 to 90 infested plants per m² and correlated with the subsequent soil-borne infection of 'Antonius' with $r = 0.66$ and 'Capo' with $r = 0.37$. The correlations also demonstrate a spread of spores from contaminated plots to previously non contaminated ones, as plots with zero infested plants show partly remarkable infestation levels in the following wheat crop. After harvest soil cultivation was done using a cultivator and a rotary harrow across the direction of sowing. In total, the amount of infested plants in the field (96 plots) before threshing was 3830 and the resulting infestation by soil-borne infection was 5725, corresponding to a factor of approximately 1.5. In that year the infection rate of 'Capo' with 3877 infested plants was 2.1 fold higher than in 'Antonius' with 1848 infested plants. The difference to the first year can be attributed to the higher initial infestation and the consequently broader and more homogeneous spore distribution in the plots, which provided better test conditions.

These results confirm the different susceptibility of 'Capo' and 'Antonius' to *Tilletia caries* also concerning the soil-borne infection cycle, which has not been proven before under Austrian conditions. Therefore, the selection of wheat variety is one important possibility to deal with soil-borne infections of common bunt. Besides crop rotation, already contaminated fields could be cultivated with resistant and/or low susceptible varieties. Nevertheless, the management of common bunt needs multiple approaches, because the fungus often mutates to new strains, which could attack upto then resistant varieties (MATHRE 2000). Apart from prevention strategies, measures need to be developed, which are adapted to regional sites and cultivation conditions in the main wheat growing regions of Austria.

Viability of spores

The spore survival of *Tilletia caries* in soils may take up to five years (BAUER *et al*. 2013). The survival is influenced by soil properties and environmental conditions like weather. It is known that dry soil conditions preserve the spore viability. In the pot trial the spore viability was tested in different substrates and moisture contents. It was assumed that the decrease of spores in the soil is influenced by microbial diversity beside soil properties and the moisture content. Figure 5 shows that spore viability in compost substrate 1 decreased more slowly in the first weeks in comparison to the soil substrate and compost substrate 2. After eight weeks the viability in all substrates declined to levels between 0.3 and 2.9%. The different moisture contents didn't influence the decrease of viability. Both moisture contents can be regarded as relatively high and may explain why no differences were found. The results also showed no significant ($p = 0.3$) differences between compacted or non-compacted substrats at both moisture contents.

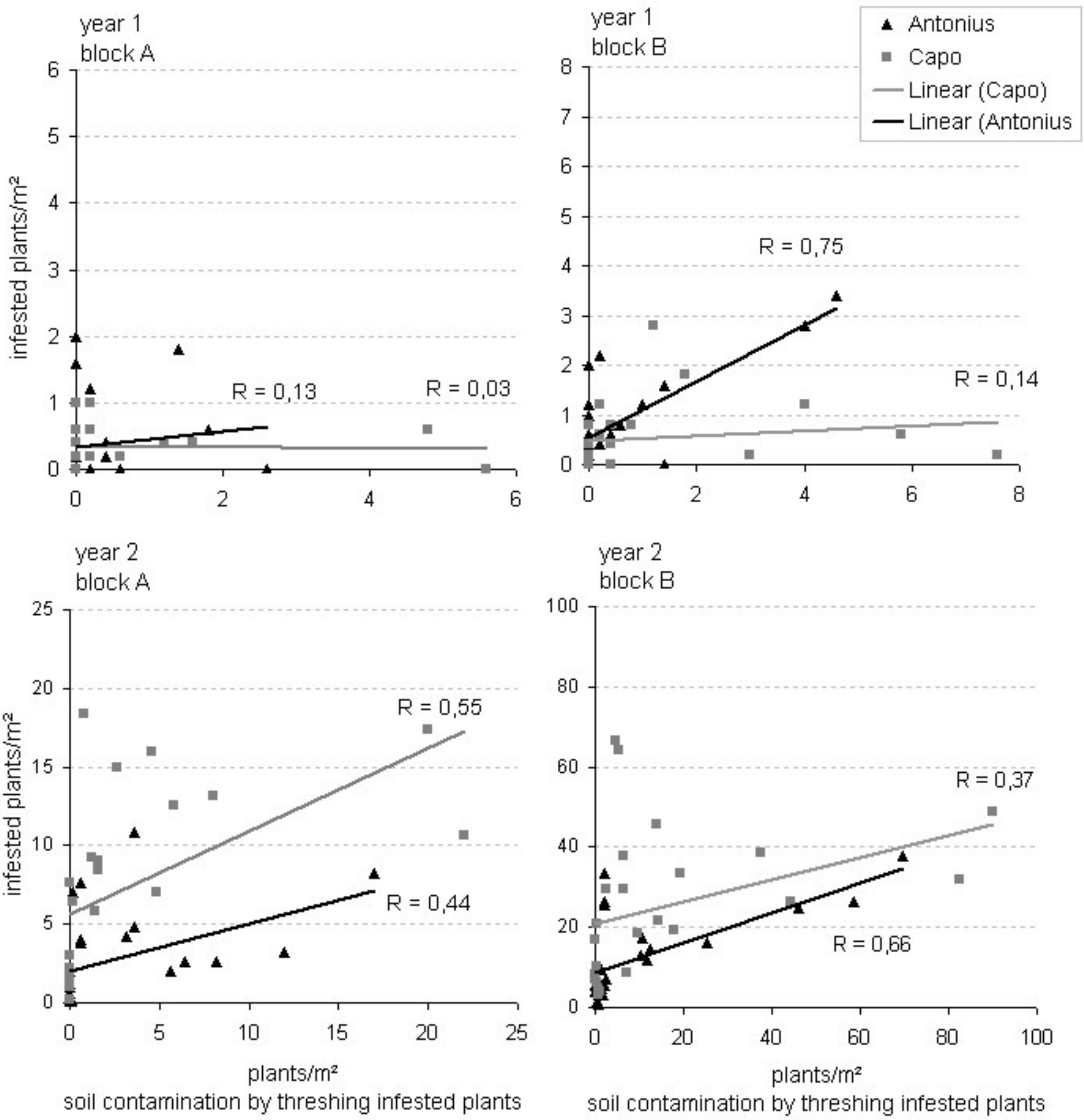

Figure 4: Infestation of winter wheat varieties 'Antonius' and 'Capo' with common bunt (*Tilletia caries*) in a two year field trial in Großnondorf. Relation between initial infestation of wheat plots by soil contamination with spores at threshing and the resulting soil-borne infection level in the following wheat crop (R, correlation coefficient; trial in each year with two main blocks A and B which differentiated in their initial infestation levels)

In the experiment with buried bunt balls the objective was to find out how long the spores are able to infect seed under field conditions. The environmental conditions in this experiment were exceptional dry from July to September 2015. In this context it was interesting to find a rapid decrease of spore viability. Figure 6 shows the initial spore viability of 71.5% and the decrease in the soil within 11 weeks to 0.7% viability. After the extraction of the bunt balls the spores were used to inoculate certified seeds which was subsequently cultivated in three plots. Two infested plants were found in the plot with spores that have been remaining for three weeks in the soil. One infested plant in each plot was found in the variants with spores that have been remaining for 7 and 11 weeks in the soil. In spite of the decreased viability of the spores after 11 weeks it can be assumed that a small percentage of viable spores is still enough to infect seeds in a degree that presents a relevant risk for spreading the disease. The same period of time, 11 to 13 weeks, was given between threshing and the following sowing of wheat at Großnondorf where remarkable infestation rates were determined.

The results from the project CARIES clearly confirmed the soil-borne infection of *Tilletia caries* under Austrian growing conditions and the potential risks of spreading the disease. Rising awareness about infection risks was, therefore, an important issue in the frame of the project. This was done by seminars and also by training farmers to recognize bunt infection in the crop and, thereby, avoid the spread of spores during harvest and associated problems with the marketability of their harvest.

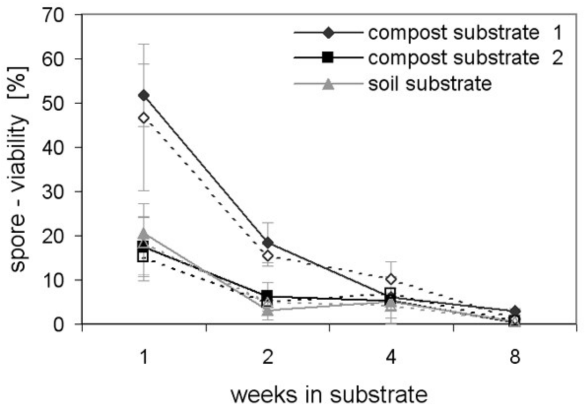

Figure 5: Viability of spores of common bunt (*Tilletia caries*) in pot trials over a period of 1 to 8 weeks with three different substrates at moisture contents 70% (continous line) and 85% (dotted line) water holding capacity

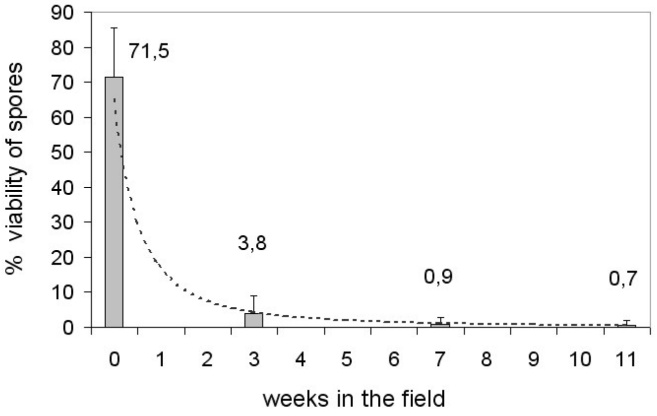

Figure 6: Viability of spores of common bunt (*Tilletia caries*) after remaining in the soil of the field over a period from 3 to 11 weeks. The 'control' treatment, *i.e*. 0 weeks, was not in the soil and demonstrates the initial viability of spores

Acknowledgements

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2016, HBLFA Raumberg-Gumpenstein, Irdning, Österreich

Influence of crop rotation, manure application and mustard catch crop on the spore potential of common (*Tilletia caries*) and dwarf bunt (*T. controversa*) in the soil

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Abstract

In organic farming common (*Tilletia caries*) and dwarf bunt (*T. controversa*) of wheat are serious diseases which cause high damages in wheat production. In case of a heavy infestation, the harvested crop can be used neither for food nor feed and additionally the soil is strongly infested with bunt spores for several years. Therefore, infestation with common and dwarf bunt of wheat has other consequences for crop production than the infestation with *e.g.* powdery mildew. During harvest, bunt spores contaminate the soil. In the soil, common and dwarf bunt spores are viable for 6 and 10 years, respectively. The spore potential in soil causes problems in subsequent wheat cultivation, as the infection also takes place from the spores in the soil. This research project mainly focused on the questions if different crop rotation systems, the application of manure as well as the cultivation of mustard as catch crop (biofumigation) play a role in the change, *i.e.* in the decrease of the number of spores in soil.

The spores in the soil were extracted by a combination of wet sieving and sedimentation steps and finally determined under a microscope according to the slightly modified ISTA method. 10 g soil of each sample were analysed. The field experiments were carried out in a split block design with 4 replicates, 10 m^2 plots, and at 3 sites (2 common bunt sites in Franconia, 1 dwarf bunt site in Upper Bavaria). The duration of the field trials were 3 years for common bunt and 4 years for dwarf bunt. Altogether 8 different crop rotation systems were investigated and finally compared. The different crop rotations were designed as follows: rotation system 1 was fallow, that means without any crop during the experimental period. This is not really a part of a crop rotation but it should be examined if a permanent open soil does influence the spore potential in soil. Rotation system 2 to 4 contained grass-clover with varying amounts, *i.e.* one, two or three year grass-clover on the plot. Rotation system 5 to 8 contained cereals with grain legumes (peas and vetches).

All rotation systems were cultivated twice, once with manure application and once without manure application. The manure application was done just once by hand at the beginning of the project. The applied amount was about 30 tons per hectare. Mustard was used as catch crop because of the high content of glucosinolates. From the literature it is known that glucosinolates prevent the germination of bunt spores. The mustard was chopped and mixed with the soil by using a rotary cultivator in order to set these glucosinolates free.

In the case of common bunt the range of the decrease of the spore potential in soil across all eight crop rotations was between 85 and 93%. Crop rotations with less or missing soil cultivation showed a significantly lower decrease of the spore potential. Due to soil cultivation the spores are brought to the surface and the spores start to germinate regardless of whether a host plant is there or not. As a result the spore potential in soil decreases. Similar results were obtained for dwarf bunt. Significant differences became evident only after 4 years of field experiments, not between all crop rotation systems but between the crop rotations “peas - winter wheat - winter rye - vetches” as well as “fallow”. In crop rotations with less soil cultivation a tendency could be observed that the decrease of the spore potential in soil is slower.

All plots with and without manure application at the “common bunt sites” over all years were compared. Plots with manure application showed a significant higher decrease of the spore potential in soil. The reason for that is the higher biological activity in soil after manure application. At the “dwarf bunt site” no significant difference in the decrease of the spore potential in soil could be observed. Dwarf bunt spores are probably more robust. In this context it is important to mention that dwarf bunt spores are viable for about 10 years in soil, whereas common bunt spores are just viable for about 6 years.

Mustard catch crop did not grow similar at all sites. The growth was less at the dry sites in Franconia in opposite to the more humid site in Upper Bavaria. Partly deficiency in nitrogen could be observed in the experimental fields. Mustard catch crop was cultivated prior to a summer crop and after peas as well as prior to winter wheat. By comparing the plots with and without mustard catch crop no signifcant difference in the decrease of the spore potential could be detected, but a slight tendency could be seen. There are two reasons why biofumigation did not take place optimally in these experiments. Firstly in autumn the soil temperature

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was considerable below the optimal temperature of 20°C and secondly mustard catch crop did not reach the main blooming period. Optimal biofumigation can be achieved by cultivating the mustard as full season crop not as catch crop but this is not an economical measure in crop production in organic farming.

Keywords

Bunt spore potential · crop rotation systems · glucosinolates · manure application · soilborne disease · *Tilletia* · *Triticum aestivum*

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2016, HBLFA Raumberg-Gumpenstein, Irdning, Österreich

Multilocal resistance assessment against common bunt of wheat (*Triticum aestivum*)

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Abstract

Bunt is one of the most devastating diseases of wheat, in Europe and Northern America mainly caused by *Tilletia caries* and *T. controversa,* in the warmer climates of the Near East by *T. foetida*. The generally obligate biotrophic pathogen is transmitted by contaminated seeds or can persist in the soil. Infection occurs at the very first moments after germination of the grain. Once penetrated into the plant, the fungus grows endophytically, remaining undetected until early maturity stages when grains are replaced by spores. Besides yield losses, the malodorant spores contaminate the grains impairing their use as seeds or as food, thereby leading to serious economic losses.

Usually the disease is controlled by the use of seed dressings and the use of certified seeds. For organic farming, however, resistance of wheat varieties to bunt is crucial as seed treatments with chemical pesticides are not allowed and several proposed alternatives, *e.g.* treatments with bacteria, hot water, vaccum-steam, steam-ultrasound or electrons, are expensive and lack efficiency. Major and some minor genes conferring resistance to *Tilletia* infections are described, however, the performance and stability and, therefore, usefulness of these resistances are under debate.

Common bunt resistance is based on Flors' gene for gene principle with an effector of the pathogen and a resistance gene in the host plants, able to detect the effector and to unleash the appropriate resistance mechanisms. To efficiently use this type of resistance, it is important to characterize the effectors in the pathogen population as well as to monitor the presence and the efficacy of the resistance genes. While many of this information is available at local and regional level, only little is known at an interregional or even continental dimension.

In order to obtain a better overview on the efficacy of resistance genes and the presence and distribution of pathogen races, the European *Tilletia* ringtest (ETR) was established including also the USA and Iraqi Kurdistan. The ringtest consists of a set of 65 wheat accessions including differentials to characterize the pathogen strains and 40 modern varieties and landraces with specific resistance features. The ringtest took place in 2015 and 2016.

First results display a wide diversity of pathogen strains, allowing to recommend the deployment of the most appropriate resistance genes in the different cropping areas. Important differences in disease severity were observed among sites. Resistance was not always linked to the postulated resistance genes. Across all test sites no susceptible plant was observed in PI636170, a breeding line selected from a Turkish landrace. Low infection levels (≤25%) were observed in some breeding lines/genetic resources and also in released cultivars, e.g. Stava and SW Magnifik.

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Keywords

Genotype by environment interaction · pathogen races · resistance genes · *Tilletia* · *Triticum aestivum*

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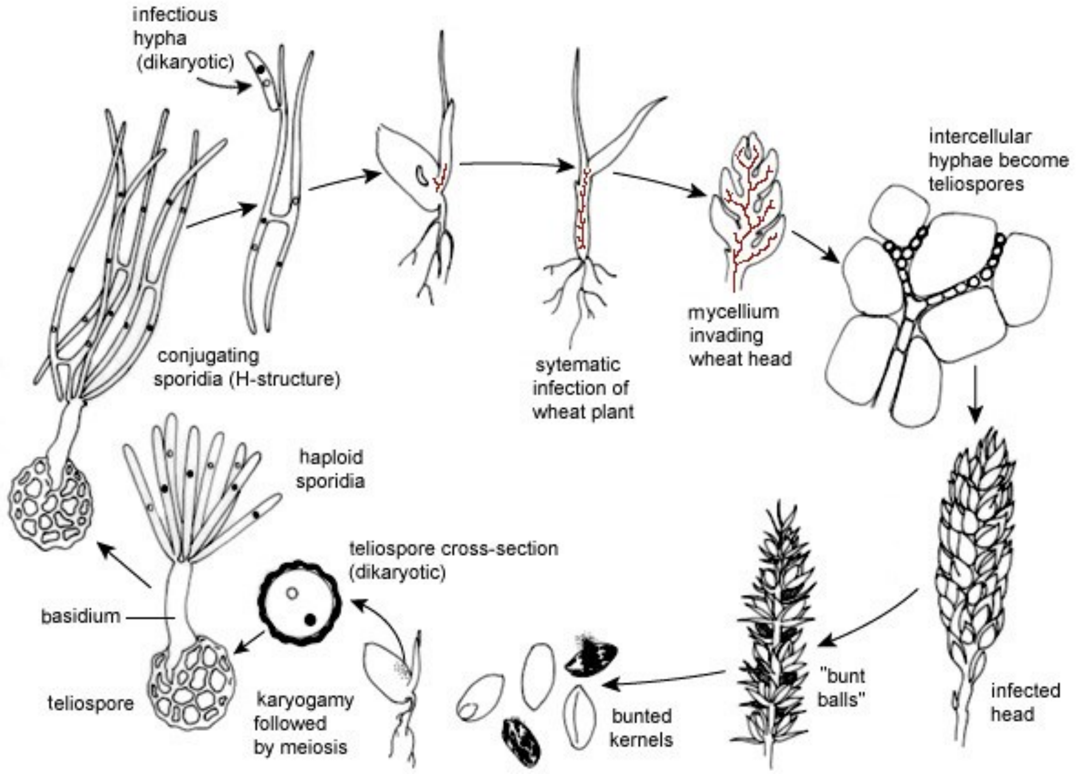

Figure 1: Disease cycle of common bunt. Survive between growing seasons is guaranteedby teliospores on the surface of healthy seed or in the soil where they can remain viable in for a number of years (Source: MATHRE 2005)

67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
21.-23. November 2016, HBLFA Raumberg-Gumpenstein, Irdning, Österreich

Comparative mapping of common bunt and dwarf bunt resistance QTL in winter wheat

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Abstract

Common bunt (*Tilletia tritici*) and dwarf bunt (*Tilletia controversa*) are devastative diseases of wheat (*Triticum aestivum* L.) that greatly reduce grain yield and quality. During the last two decades bunt diseases have re-emerged throughout Europe due to the steep increase in organic winter wheat production which cannot make use of chemical seed-treatments – and a complete lack of adapted and resistant varieties. To large extents, the genetic basis of bunt diseases is still unknown: Few of the 14 bunt resistance genes (*Bt1* to *Bt13* and *BtP*) and QTL were mapped to specific chromosomes in winter wheat; Molecular markers that find use in marker assisted selection, however, exist for *Bt10* solely. Therefore, the identification of major bunt resistance QTL and closely linked molecular markers will help to speed up the development of bunt resistant varieties for organic farming.

Three bi-parental recombinant inbred line (RIL) populations were derived from crosses of the bunt resistant North-American cultivars 'Blizzard' and 'Bonneville' – both carriers of unknown bunt resistance genes, and 'PI 119333' – a Turkish landrace and carrier of the highly effective bunt resistance gene *Bt12*, with the susceptible cultivar 'Rainer'. Phenotypic reaction of all RIL populations, each comprising 80-120 lines, to artificial inoculation with common bunt and dwarf bunt teliospores was evaluated in five trials over three years at two locations in Austria and the USA. In addition, all RIL populations were genotyped by single nucleotide polymorphism (SNP) markers using the Illumina 15K array.

The combined statistical analysis of phenotypic and genotypic data allowed us to identify major QTL for common bunt and dwarf bunt resistance in winter wheat that explained large amounts of the total phenotypic variation (R^2): For 'Blizzard' and 'Bonneville', two major QTL were found on wheat chromosome 1A ($R^2 = 20$ and 28%, respectively) and 1B ($R^2 = 30$ and 35%, respectively); While the QTL found on 1A conferred common bunt and dwarf bunt resistance, the one on 1B conferred common bunt resistance solely. For PI119333, a carrier of the bunt resistance gene *Bt12* which confers highly efficient protection against common bunt and dwarf bunt across countries, one major bunt resistance QTL (i.e. *Bt12*) mapped to chromosome 7D and explained around 40% of the total phenotypic variation. The identified major bunt resistance QTL on wheat chromosomes 1A, 1B and 7D and associated SNP markers will find application in marker assisted selection and accelerate the development of bunt resistant varieties for organic agriculture.

Keywords

Bt resistance genes · genotyping · organic breeding · seedborne disease · *Tilletia* · *Triticum aestivum*

Acknowledgements

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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Evaluation of resistance against common bunt in spelt wheat

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Abstract

Spelt wheat (*Triticum spelta* L.) is an old domesticated species. Spelt is often considered to be a valuable genetic source of desirable genes. An increasing attention is payed to spelt with respect to production of healthy and organic food products. Common bunt, caused by *Tilletia caries* (syn. *T. tritici*) and *T. laevis* (syn. *T. foetida*), reduces yield and quality in organic as well as in conventional production. Genetic resistance represents a promising tool of control of common bunt of spelt wheat in low input and organic farming conditions, where the possibilities of seed treatment are limited. Within the framework of the HealthyMinorCereals project, the resistance to common bunt, together with leaf rust, yellow rust, stem rust and Fusarium head blight resistance was evaluated. In total, 80 genotypes of winter spelt wheat of different origin were included in the tests. The contribution presents data on common bunt resistance of the evaluations from 2015 and 2016. Results from the Czech Republic, Austria and Switzerland revealed the highest resistance level against common bunt in genotypes 'Albin' and 'Sofia 1'.

Keywords

Genetic resources · hulled wheat · organic agriculture · seedborne disease · *Triticum spelta*

Introduction

Spelt wheat (*Triticum spelta* L.) is considered to be an old cultivated European wheat species. Spelt's popularity has been rising in recent years, especially with regard to its nutritional value, digestibility and taste. This is also a reason, why more attention is paid to the health condition of spelt. Spelt wheat is attacked by the same diseases and in a similar way as common wheat (*T. aestivum* L.), nevertheless it is generally considered to be more resistant. Common bunt control has a significant importance for spelt cultivation, as it is mostly cultivated in organic farming or in low input systems. Protection against harmful organisms in organic farming is based especially on a good cropping practice, growth morphology and selection of crop species. With regard to the fact that the use of chemical protection is limited in organic farming, it is necessary to pay appropriate attention to the utilisation of resistance sources.

Tilletia caries (D.C.) Tul. & C. Tul. (syn. *T. tritici* (Bjerk.) G. Winter and *T. laevis* J.G. Kühn (syn. *T. foetida* (Wallr.) Liro may cause serious damages due to the decrease of crop yield and quality. In case of heavy incidence it is not possible to use the seed as food or feed. Already low doses of the spores represent a problem for seed sales and multiplication. The spores contain trimethylamin causing an unpleasant odour.

In field tests with artificial infection of common bunt strains that are maintained at the Crop Research Institute in Prague, we usually encounter a high bunt incidence in registered winter wheat varieties (DUMALASOVÁ & BARTOŠ 2016). Resistance to common bunt in European wheat varieties is rather seldom. For spelt wheat, there is not much information available on resistance to common bunt (HE & HUGHES 2003).

Material and methods

A panel of 80 genotypes of winter spelt wheat was established within the framework of the FP7 project HealthyMinorCereals and evaluated for the resistance to common bunt. Additionally, 23 winter wheat cultivars registered in the Czech Republic were tested.

Field tests with artificial inoculation of common bunt were carried out in Prague, Czech Republic (Crop Research Institute), Tulln, Austria (BOKU-University of Natural Resources and Life Sciences, Vienna) and Stäfa, Switzerland (Getreidezüchtung Peter Kunz, Feldbach). The inoculum was a mix of strains of common bunt of local prevenience. Results from the first two years of testing are available so far.

Field trials in Prague had two replicates, each of them represented by one row of 1 m length; distance between rows was 0.2 m. Seed was inoculated with a mixture of common bunt teliospores before sowing. Inoculation was done by shaking 250 seeds with 0.1 g of teliospores in Erlenmayer flasks for 1-2 min. Inoculations and sowing were carried out by hand in October. The methods applied on the two other localities were adapted to their specific conditions. In Stäfa seeds were dehulled before sowing.

The total amount of spikes and total amount of infected spikes per replicate was counted in July. The reaction to bunt was expressed as a percentage of spikes exhibiting bunt. For the identification of races an infection incidence above 10% of the spikes indicates virulence (GOATES 1996). On the basis of this rule we assume that the breeding potential of genotypes showing more than 10% of infection is low.

The same set of genotypes was evaluated also for reaction to leaf rust, yellow rust, stem rust and Fusarium head blight. The reaction to rusts quoted in this contribution was determined as described by HANZALOVÁ & BARTOŠ (2014).

Dumalasová V, Hanzalová A, Bartoš P, Chrpová J (2017) Evaluation of resistance against common bunt in spelt wheat. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 67. Jahrestagung 2016, 21-23 November, Raumberg-Gumpenstein, pp 41-45. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-45-9

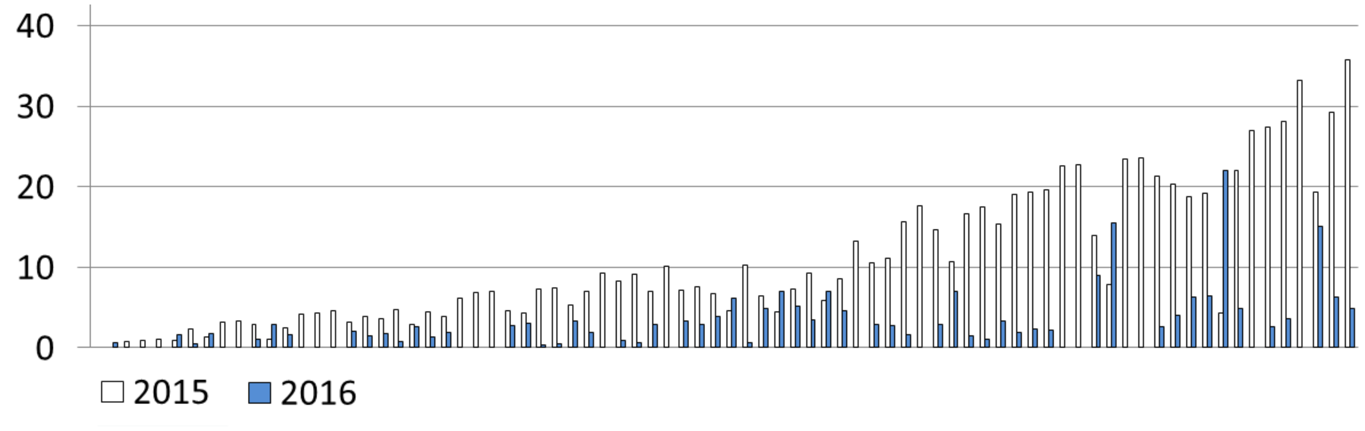

Figure 1: Common bunt infection (% infected spikes) of 80 *Triticum spelta* genotypes in 2015 and 2016 in Prague, Czech Republic. Artificial inoculation was carried out on hulled seeds.

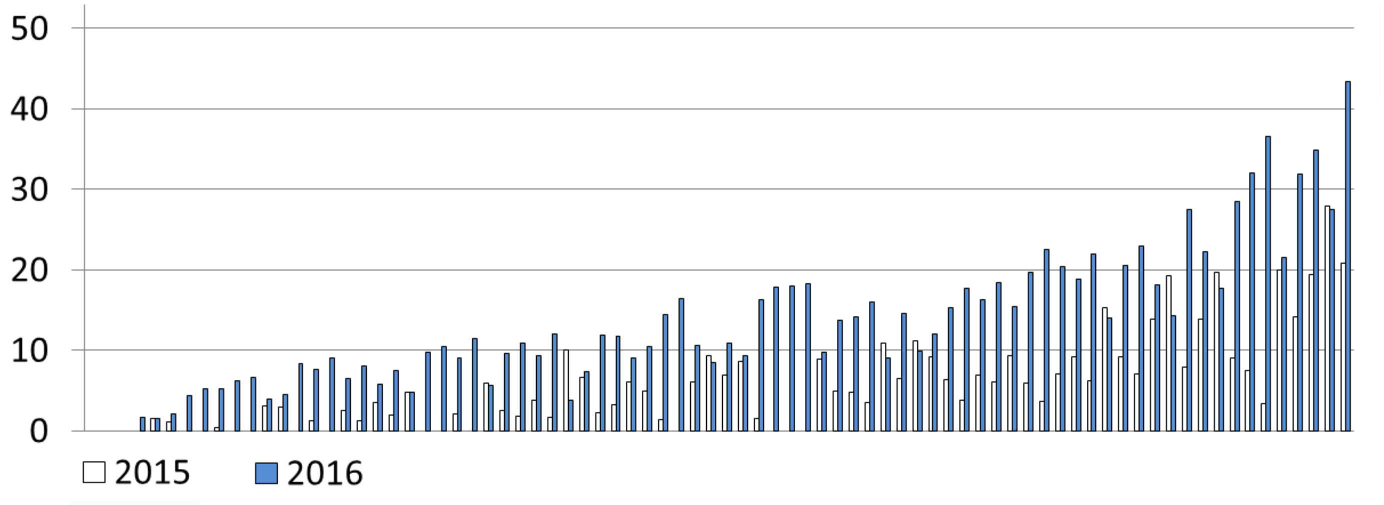

Figure 2: Common bunt infection (% infected spikes) of 80 *Triticum spelta* genotypes in 2015 and 2016 in Tulln, Austria. Artificial inoculation was carried out on hulled seeds.

Results and discussion

The level of bunt incidence in the replications corresponded to each other well with respect to the localities Prague and Tulln in both years and most cases. Significant differences were rare and they appeared mainly if the level of bunt infection on the locality in the year was not sufficient.

From the tests in Prague and Tulln the fluctuations in the level of bunt incidence due to various conditions of the environment in the different years could be determined.

Common bunt infection observed in field trials at Prague-Ruzyně in 2015 and 2016 is shown in Table 1. The bunt incidence obtained in 2015 in Prague was sufficient. The mean value was 10.7% and the maximum value reached 35.7% of infected spikes.

The level of infection in Prague was lower in 2016 (Figure 1), with 2.7% as mean and 22.0% as maximum. The reason for the lower infection in 2016 was most probably the unfavorable climatic conditions in autumn 2015, i.e. warm and dry weather.

Contrary to Prague, in Tulln the bunt incidence was lower in 2015 with a mean value of 5.8% and a maximum of 27.9%. In 2016, the level of infection in Tulln was similar to Prague in 2015 with a mean of was 13.5% and a maximum of 43.4% (Figure 2).

For Stäfa a significant higher bunt infection was recorded. Mean infection in 2016 was 53.7% and the maximum was 94.9% (Figure 3). This really high level of infection can be explained by the different infection method. Whereas in Prague and Tulln hulled seeds were inoculated, dehulled seeds were inoculated in Stäfa. Hence, it is obvious that the presence of glumes in hulled seeds of spelt wheat is responsible for a lower level of infection. This finding is in accordance with former results from tests with hulled and dehulled wheat genotypes (DUMALASOVÁ & BARTOŠ 2010).

The seed may present a mixture of hulled and dehulled seeds. Their ratio influences the level of infection, which could be lower when more seeds are covered with glumes. The fact that dehulling removes a part of the spores together with the glumes and contributes to a decrease of infection is important for practice. The presence or absence of glumes has probably a lower effect on the infection of plantlets with dwarf bunt because of the mainly soil-borne origin of the inoculum of this pathogen.

Compared to the hulled spelt wheat, naked common wheat was more susceptible to common bunt in the 2015 and 2016 tests in Prague. The mean level of infection of 23 common wheat cultivars currently registered in the Czech Republic was 30.8% in 2015 (maximum 47.5%) and 14.4% in 2016 (maximum 51.6%) (Figure 4).

Table 1: Mean levels of common bunt infection observed in field trials in Prague in 2015 and 2016

Genotype	% bunted ears			Genotype	% bunted ears		
	2015	2016	Mean		2015	2016	Mean
ALBIN	1.0	1.6	1.3	NEUEGGER WEISSKORN	0.0	0.6	0.3
ALKOR	7.0	2.9	5.0	OBERKULMER ROTKORN	7.6	2.9	5.3
ALTGOLD	9.3	3.4	6.4	OEKO 10	7.4	0.5	4.0
BADENGOLD	7.3	5.1	6.2	OSTRO	4.1	0.0	2.1
BADENKRONE	23.5	0.0	11.7	POEME	3.3	0.0	1.6
BADENSTERN	6.4	4.9	5.6	RINIKER WEISSKORN	14.7	3.0	8.8
BLACK FOREST	26.9	0.0	13.5	ROSÉN	17.6	0.0	8.8
BURGDORF WEISSKORN	33.2	0.0	16.6	ROTER SCHLEGEL DINKEL	6.2	0.0	3.1
BURGHOF	7.0	0.0	3.5	ROTTWEILER DINKEL ST.6	2.9	2.7	2.8
CERALIO	7.1	1.9	4.5	ROTTWEILER FRÜHKORN	7.3	0.4	3.8
COSMOS	19.7	2.2	10.9	ROUQUIN	6.9	0.0	3.4
EBNERS ROTKORN	9.3	0.0	4.6	RUBIOTA	11.1	2.7	6.9
ELSENEGGER	20.2	4.0	12.1	RUEFENACHTER WEISSKORN	19.0	2.0	10.5
EPANIS	10.6	2.9	6.7	SALEZ	15.3	3.3	9.3
FARNSBURGER ROTKORN	4.5	7.0	5.7	SAMIR	35.7	4.9	20.3
FILDERSTOLZ	3.2	0.0	1.6	SCHAFFISHEIM WEISSKORN	2.9	1.1	2.0
FRANCKENKORN	3.1	2.1	2.6	SCHNOTTWILER WEISSKORN	5.4	3.3	4.3
FRIENISBERGER WEISSKORN	10.3	0.6	5.4	SCHWABENSPELZ	19.4	15.1	17.2
FUGGERS BABENHAUSENER ZUCHTVEESEN	2.4	0.4	1.4	SOFIA 1	0.0	0.0	0.0
GOLDIR	6.8	3.9	5.4	SPY	4.6	2.7	3.6
GUGG 11A	16.7	1.4	9.1	STRICKHOF	22.1	4.8	13.4
GUGG 2F	1.3	1.7	1.5	T. SPELTA RUZYNE SVTLA	19.2	6.4	12.8
GUGG 2G	1.1	2.9	2.0	T. SPELTA ALBUM	27.4	2.6	15.0
GUGG 4E	3.8	1.4	2.6	TAURO	13.3	0.0	6.6
GUGG 4H	4.4	22.0	13.2	THUERIG ROTKORN	28.1	3.6	15.8
GUGG 5A	10.7	7.0	8.9	TITAN	22.5	0.0	11.3
GUGG 5C	4.3	3.1	3.7	TOESS 5B	7.1	3.3	5.2
GUGG 6A	0.9	0.0	0.4	TOESS 6D	19.3	2.3	10.8
GUGG 9A	4.6	6.1	5.4	T. SPELTA KROMERIZ	0.8	0.0	0.4
GUGG 9F	2.5	1.6	2.0	VON RECHBERGS BRAUNER WINTERSPELZ	4.4	1.3	2.9
H57-7	5.8	7.1	6.4	VÖGELERS	17.5	1.0	9.3
HERCULE	4.4	0.0	2.2	VON RECHBERGS FRÜHER WINTERDINKEL	29.3	6.2	17.8
HOLSTENKORN	3.6	1.7	2.7	VORENWALDER WEISSKORN	15.7	1.6	8.6
HUESLERS-NIEDERWIL 19	8.3	1.0	4.6	WAGGERSHAUSER WEISSER KOLBEN	18.7	6.4	12.5
LANTVETE FRAN GOTLAND	21.4	2.6	12.0	WILLISAUER WEISSKORN	9.2	0.6	4.9
LIESTALER ROTKORN L11	7.8	15.5	11.6	WINIGER-EGG WEISSKORN	22.7	0.0	11.4
LONIGO	14.0	9.1	11.5	ZEINERS WEISSER SCHLEGELDINKEL	23.6	0.0	11.8
LW 12 NUERTINGEN	3.9	1.9	2.9	ZOLLERNSPELZ	4.7	0.8	2.7
LW 13 NUERTINGEN	4.6	0.0	2.3	ZÜRCHER OBERLÄNDER ROTKORN	8.6	4.6	6.6
MURI ROTKORN	1.1	0.0	0.5	ZUZGER	10.2	0.0	5.1

Table 2 shows the most susceptible genotypes from the trials performed in Prague, Stäfa and Tulln, *i.e*. 'Strickhof', 'Vögelers', 'Von Rechbergs Früher Winterdinkel', 'Black Forest', 'Cosmos', 'Lantvete fran Gotland', 'Samir' and 'Schwabenspelz'. The absence of effective resistance genes to bunt was most obvious in the dehulled variant tested in Stäfa, where the most susceptible genotypes had 68.5% - 88.5% infected spikes. Variability of bunt infection was higher when the hulls were still present at artificial inoculation; in this case a bunt incidence below 10% was more frequent.

The most resistant genotypes (Table 3) were 'Sofia 1', 'Albin', *T. spelta* Kromeriz, 'Gugg 2G', 'Ostro', 'Altgold', 'Ceralio' and 'Spy'. Also in this group some variation occurred. For some genotypes in some years and localities the bunt incidence exceeded 10%. Such genotypes are not suitable sources of resistance. For resistance breeding the genotypes with zero bunt incidence in the infection tests are desired. It is not clear, whether the varieties with varying bunt incidence escaped infection in some cases or whether specific resistance genes to the used inoculum strains are present.

Genotypes 'Sofia 1' and 'Albin' had a very low bunt incidence both in the hulled and dehulled variant. 'Sofia 1' had also an intermediate resistance to stem rust, while the other spelt wheat genotypes showed high susceptibility to stem rust in the field test, and was the most resistant genotype to leaf rust in the both test years.

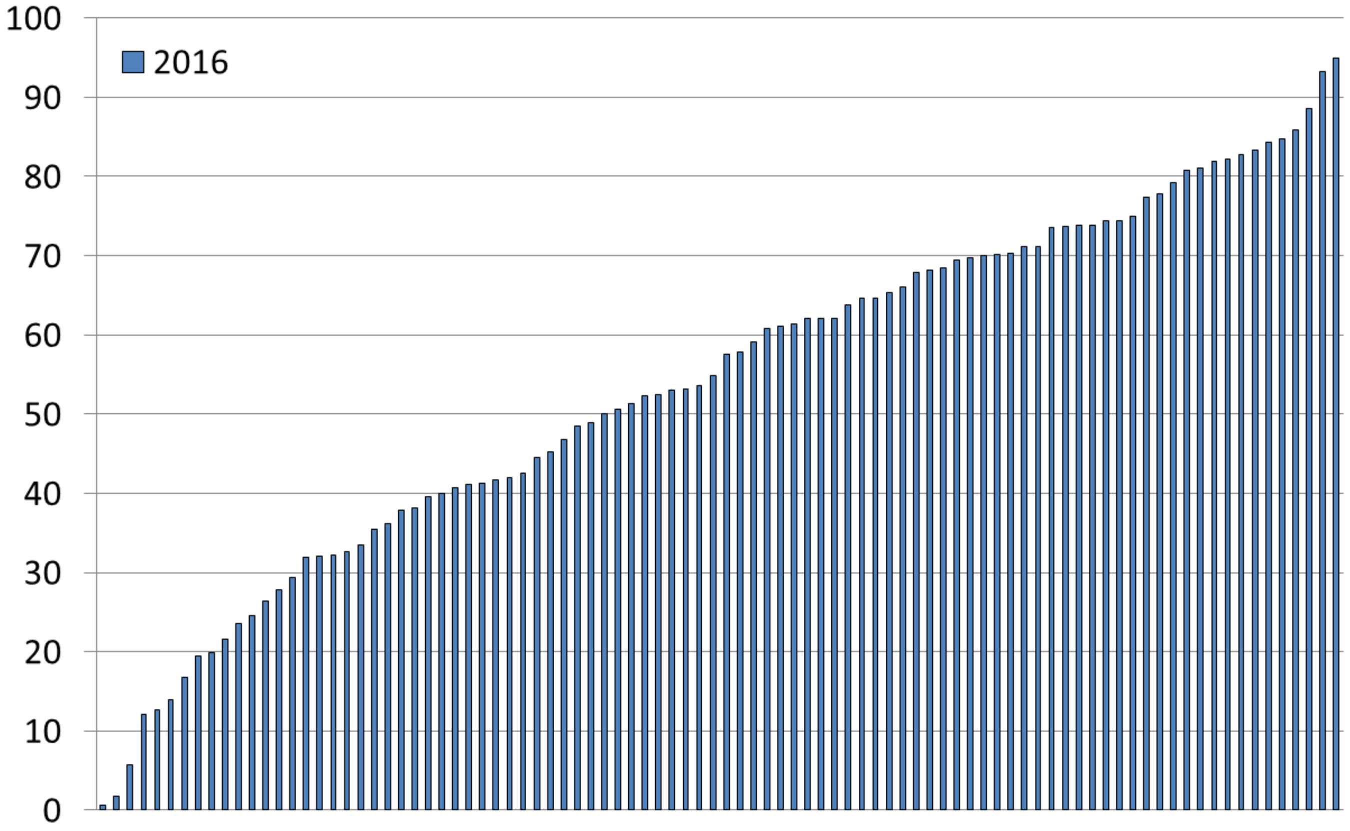

Figure 3: Common bunt infection (% infected spikes) of 80 *Triticum spelta* genotypes in 2016 in Stäfa, Switzerland. Artificial inoculation was carried out on dehulled seeds.

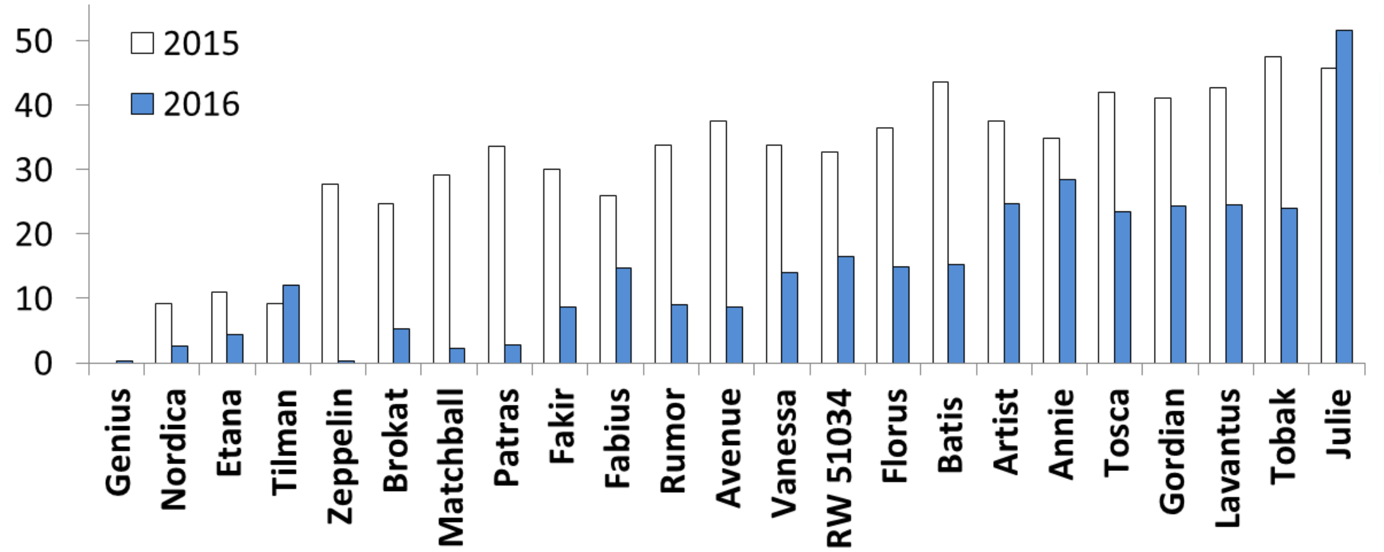

Figure 4: Common bunt infection (% infected spikes) of 23 *Triticum aestivum* cultivars currently registered in the Czech Republic in 2015 and 2016 in Prague, Czech Republic.

Table 2: Mean levels of common bunt infection observed on the most susceptible spelt wheat genotypes in field trials in the Czech Republic, Austria and Switzerland in 2015 and 2016

Genotype	% bunted ears				
	Stäfa 2016	Prague 2015	Prague 2016	Tulln 2015	Tulln 2016
STRICKHOF	83.3	22.1	4.8	9.1	12.0
VÖGELERS	84.3	17.6	1.0	13.8	18.1
VON RECHBERGS FRÜHER WINTERDINKEL	85.8	29.2	6.2	9.2	8.5
BLACK FOREST	68.5	26.9	0.0	14.1	31.9
COSMOS	88.5	19.6	2.2	9.0	28.5
LANTVETE FRAN GOTLAND	80.8	21.4	2.6	27.9	27.5
SAMIR	77.9	35.7	4.9	19.3	34.8
SCHWABENSPELZ	81.9	19.4	15.1	20.8	43.4

Table 3: Mean levels of common bunt infection observed on the most resistant spelt wheat genotypes in field trials in the Czech Republic, Austria and Switzerland in 2015 and 2016

Genotype	% bunted ears				
	Stäfa 2016	Prague 2015	Prague 2016	Tulln 2015	Tulln 2016
SOFIA 1	1.7	0.0	0.0	0.0	0.0
ALBIN	0.6	1.0	1.6	0.0	0.0
T. SPELTA KROMERIZ	12.1	0.8	0.0	1.1	2.1
GUGG 2G	16.8	1.1	2.9	0.0	5.2
OSTRO	5.7	4.1	0.0	0.0	16.5
ALTGOLD	14.0	9.3	3.4	3.1	3.9
CERALIO	24.6	7.1	1.9	0.0	1.7
SPY	19.5	4.6	2.7	0.0	11.5

Conclusion

Based on these results from the Czech Republic, Austria and Switzerland, 'Albin' and 'Sofia 1' can be considered as valuable resistance sources to common bunt. As far as we know, the resistance genes of these two genotypes haven´t been characterized yet.

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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Elucidation of seed, rhizosphere and phyllosphere microbiomes – Implementations for biocontrol and plant breeding

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Abstract

Advancements in sequencing technologies and microscopic techniques allow gathering valuable information about the genetics and ecology of plant-associated microbiota. Insights reveal a co-evolution of plants and their microbiota based on intimate interactions in essential functional contexts. They stimulate germination and growth, defend disease, protect against stresses, and improve plant performance. The rhizosphere microbiome plays a crucial role for plant host performance and due to vertical transmission of core microbiomes, the seed microbiomes contribute to the rhizosphere assembly. Additionally, functional features supports differences within habitats and suggests that seed microbiomes serve as a reservoir for features essentially needed for rhizosphere functionality. We embrace the entire plant system including seeds and rhizospheres, which are ideal initiation sites for precise microbiome control. The generation-spanning transmission of specific and beneficial microbiota is essential for stability and a key component in ecosystem and plant health. The comprehensive understanding and connectivity of plants and their microbiota suggest that integrated microbiomes in plant breeding can contribute to new management strategies in modern sustainable agriculture and further lead to the development of so-called next-generation bio-products for disease control and plant growth promotion with stable field efficacy.

Keywords

Biological control · plant-microbiome interaction · rhizosphere microbiome · seed microbiome · seed protection · seed treatment

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From lab to field: *Paraburkholderia phytofirmans* PsJN genome analysis and application strategies

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Abstract

The plant is habitat for a rich microbial life. Bacteria and fungi colonize the rhizosphere (soil attached to the root surface), the phylosphere (surface of plants) as well as the inner tissue of plants (endopshere). Those microorgansims colonizing the endophytere are consequently referred to as endophytes. Extensive analysis in the last decades allowed the identification of endophytes in both monocotyledonous and dicotyledonous plants, ranging from woody tree species to mosses.

Endophytes colonize an ecological niche similar to that of phytopathogens and host-plant/endophyte interactions are often considered mutualistic – the microorganisms gain nutrients and a protected niche to occupy, whereas the host benefits from bacterial activities resulting in plant growth promotion, improved nutrient uptake, increased stress tolerance, control of plant pathogens and induction of systemic resistance.

Paraburkholderia phytofirmans PsJN is a naturally occurring plant-associated bacterial endophyte that effectively colonizes a wide range of plants and stimulates growth and vitality its host plants. Whole-genome sequencing and comparative analysis revealed that the genome of PsJN consists of two chromosomes and one plasmid, well equipped with genes for the adaption to changing evironments and interaction with plants. For example, the PsJN genome contains at least two independent pathways for the production of idole-3-acetic acid (IAA), the major auxin. Also genes for the synthesis of siderophores (iron transporting compounds) and ACC deaminase genes are present in *P. phytofirmans* PsJN. The 1-aminocyclopropane-1-carboxylate (ACC) deaminase is supposed to be involved in balancing the plant ethylene levels and thus in plant protection against biotic and abiotic stress, such as temperature variations, flooding, toxins, drought or high salt as well as enhance resistance to low level of pathogens. Furthermore, strain PsJN contains numberous genes for the degradation of complex organic compounds and detoxification, *e.g.* 24 glutathione-S-transferase genes. All together the ability of *P. phytofirmans* PsJN to establish populations in diverse environments seems to be based on its large genome harboring a broad range of physiological functions.

The mechanisms of plant-endophyte communication and bacterial adaption to the plant environment are still poorly understood. We therefore performed whole-transcriptome sequencing of *P. phytofirmans* PsJN colonizing potato (*Solanum tuberosum* L.) plants to study *in planta* gene activity and the response of strain PsJN to plant stress. We inoculated *in vitro* potato plantlets with strain PsJN and after two weeks of incubation induced osmotic stress in the plants by adding polyethylene glycol. RNA was isolated from plants one, six and twelve hours after stress induction. Not stressed plants were also sampled. The transcriptome of PsJN colonizing potato plants showed a broad array of functionalities encoded in the genome of strain PsJN. More than 60% of the genes encoded on the genome of strain PsJN were expressed in planta. The data revealed that the endophytic life of bacteria does not require very selected and specialized functions. Transcripts upregulated in response to plant drought stress were mainly involved in transcriptional regulation, cellular homeostasis, and the detoxification of reactive oxygen species, indicating an oxidative stress response in PsJN. Our hypothesis is that water limitation in plants results in production of high amounts of reactive oxygen species is plants. Endophytic PsJN cells respond with the expression of genes for the defense against oxidative stress in order to prevent cell damage. Whether this could help to maintain the redox balance in plants and thus minimize the effects of drought stress in plants will be studied in future.

During the past few decades, plant growth-promoting bacteria have been well acknowledged for their use in plant protection. In order to make the potential of plant growth promoting bacteria available for application in agriculture we develop strategies to deliver viable bacterial cells to the plant, *e.g.* by producing plant seeds containing the desired bacterium. Specifically, by application of *P. phytofirmans* PsJN onto the flowers of parent plants we could drive its inclusion in seeds. We demonstrated the introduction of PsJN into seeds of monocot and dicot plant species and the consequential modifications of growth traits.

Keywords

Comparative genomics · endophytes · endoseed · strain delivery · transcriptomics

Petric A, Sheibani-Tezerji R, Pfaffenbichler N, Naveed M, Rattei T, Compant S, Antonielli L, Mitter B (2017) From lab to field: *Paraburkholderia phytofirmans* PsJN genome analysis and application strategies. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 67. Jahrestagung 2016, 21-23 November, Raumberg-Gumpenstein, pp 49-50. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-45-9

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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Microsymbionts influence resistance of field pea against *Didymella pinodes* at phenotypic and molecular levels

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Abstract

The demand for protein is increasing steadily with rapid global human population growth. In contrast, the production of protein (legume) crops such as field pea (*Pisum sativum* L.) is declining due to abiotic and biotic stresses as well as socioeconomic factors. Particularly, biotic stresses like viruses and the Ascochyta blight fungi *Didymella pinodes* are the most damaging pathogens of pea. Their attack often results in the loss of the entire crop, especially in monoculture and whenever favorable environmental conditions for their infection and further development occur. Recent evidences showed that beneficial microbes in the rhizosphere prime the whole plant for enhanced defense or resistance against a broad range of pathogens and insect herbivores and abiotic stresses. Here, we aimed to investigate the effects of rhizobia and mycorrhiza at phenotypic and bimolecular levels of pea plants against *D. pindodes* infection.

In pot experiments, we studied the priming effects of the symbionts to confer protection against the fungal pathogen *D. pinodes* in field pea. A factorial experimental design with susceptible pea cultivar 'Messire', including three symbionts (arbuscular mycorrhiza fungus, *Rhizobium* and co-inoculation of both) and a non-symbiotic control treatment, and two pathogen infection levels (with or without) were laid out in a completely randomized design with four replicates. After the pathogen infection, disease severity, metabolome and proteome of leaf and shoot biomass were analyzed.

Our results indicated that some characteristics such as dry matter production, the regulation of the TCA, amino acid and secondary metabolism including the pisatin pathway were most pronounced in the single rhizobial associated pea plants, which had also the lowest infection rate and the slowest disease progression.

In the past, reports showed that metabolic exchanges from the microbial symbionts for host carbon have a stimulatory effect on leaf photosynthetic capacity, enhance green area growth and may also trigger systemic induced resistance against stress.

In this study, the synergetic effects of the two beneficial microbes, particularly on phenotypic characters were not evidenced. That is in agreement with some previous studies, but also contradicting with other researchers who reported synergistic effects. However, the co-inoculated plants exhibited elevated abundance of stress related proteins with a concomitant adjustment of proteins involved in jasmonate synthesis.

With this study we provide new insights into the symbionts induced systemic resistance of the leaf proteome as well as the specific early pathogen stress response. We suggest further a time series infection test and field trials to better understand the effects of *D. pinodes* on the growth, development, final yield and quality of different pea genotypes.

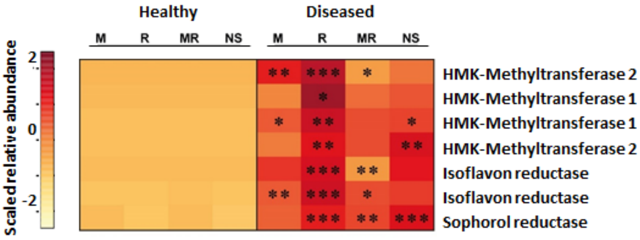

Figure 1: Microsymbionts effect on pisatin synthesis of pea plants

Keywords

Metabolome · pathogen · *Pisum sativum* · proteome · symbionts

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Cultivar specificity of the rhizosphere and seed microbiomes of the Styrian oil pumpkin

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Abstract

Collectively known as the plant microbiome, plant-associated microbes can help plants to fend off diseases, stimulate growth, occupy space that would otherwise be taken up by pathogens and promote stress resistance. Additionally they influence crop yield and quality. The specific arrangement of plant-associated microbiomes is supposed to be a consequence of breeding activities and genotype selection. To support a concept involving beneficial plant-microbe interactions in breeding activities of Styrian oil pumpkin (*Cucurbita pepo* subsp. *pepo*), the microbiomes of seeds, rhizospheres and bulk soil were interpreted.

Seeds and rhizospheres of 14 genotypes showing different morphological and horticultural characteristics as well as the bulk soil were analyzed using a 16S rRNA gene amplicon sequencing approach, which was assessed by bioinformatics and statistical methods. One open-pollinated cultivar, three oil pumpkin hybrids and their corresponding inbred lines, five segregating lines and a zucchini hybrid were evaluated.

In general, the diversity of the observed operational taxonomic units (OTUs) in rhizosphere samples was significantly higher than in seed samples. In contrast to the rhizosphere microbiomes, a strong genotype specificity was detected for the seed-associated microbial communities. The seed core microbiome of the cultivars analyzed in this study was dominated by high abundances of seven *Enterobacteriaceae*, one *Pseudomonadaceae*, one *Lactococcus* and one *Exiguobacterium* OTU. In the open-pollinated cultivar and in one inbred line, the microbiome was comprised, to a large extent, of the genus *Erwinia* as well as the important pathogen *Pectobacterium carotovorum* (syn. *Erwinia carotovora*), the causal agent of soft rot of fruits. Potential plant-beneficial bacteria like *Lysobacter*, *Paenibacillus* and *Lactococcus* contributed to the microbial communities as well. In the two three-way cross hybrids and one of the segregating lines *Firmicutes* were a prevalent group. Three cultivars showed a significant higher microbial alpha-diversity in their seeds (Shannon diversity index H´ of 8.6, 7.9 and 7.1) as well as a higher Heip evenness index E´ (0.29, 0.17, 0.16) than the other eleven genotypes investigated (average Shannon diversity index of 4.7, average Heip evenness index of 0.05). No strong relationships among the hybrids and their pedigree components could be detected. The results of the seed analysis are of particular interest for the seed production industry, as the Styrian oil pumpkin is highly susceptible to various fungal and bacterial pathogens during germination making chemical or complex seed treatments inevitable. It remains to be investigated to which extent naturally occurring seed-borne bacteria influence germination and plant development. Those results could direct the design of tailored biological seed treatments or influence seed disinfection strategies that might replace fungicide treatments in future. The genotype-specific composition of the seed microbiome should be considered in breeding of new cultivars: a possible implication for breeding programs could be the selection of genotypes enriching less enterobacteriaceal pathogens and/or expressing a higher microbial diversity in their seeds.

Keywords

16S rRNA gene amplicon sequencing · breeding · *Cucurbita pepo* · plant-microbe interaction · seed treatment

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Soybean improvement through the application of endophytes isolated from seeds

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Abstract

Soybean (*Glycine max* (L.) Merr.) is an important crop of worldwide distribution. Being the main source of world dietary protein, soybean is used mostly for cattle feeding but also in the elaboration of human food products. The increasing demand of non GMO soybean produced under organic agriculture is promoting the cultivation of this crop in Europe. To be a profitable crop, it is essential to improve the competitiveness by increasing the productivity under European climate conditions. The use of beneficial bacteria promoting the development of plants is an environmental respectful practice. The bacteria associated with plants are involved directly in several mechanisms related with the development of the plant (*e.g.* nitrogen fixation, mobilization of nutrients and production of phytohormones) and also indirectly, acting as a biocontrol agent. This comprises for example the induction of defence mechanisms of the plant and outcompeting potential pathogens. This positive relationship is especially remarkable under adverse conditions. Endophytic bacteria applied on seeds can unfold these positive effects from a very early growth stage in the plant. In the present research endophytic bacterial strains from soybean seeds have been characterized to identify potential strains that can improve the soybean crop.

Endophytic bacteria strains were isolated from surface sterilised seeds of five different soybean varieties, *i.e.* ES Mentor (E), Merlin (M), Málaga (A), PZO Silvia (P) and SY Livius (S). Bacteria strains were identified by sequencing partially the 16S rRNA and 23S rRNA genes. Strains belonging to the *Bacillus* and *Paenibacillus* genera were selected for the characterization assays as many strains of them are used with good results as plant growth promoters in other plant species. Characterization assays carried out were the germination assay under cold temperatures (12°C for 21 days); evaluation of siderophore, indol 3-acetic acid (IAA) and surfactin production, mobilization of phosphorus and evaluation of *in vitro* biocontrol capacity against *Rhizoctonia solani* and *Sclerotinia sclerotiorum*.

Several endophytic bacteria showed high potential in the improvement of soybean germination under cold conditions. Those bacteria belong to the *Bacillus* genera, and the strains P1 and S4 had the best results. P1 is not related with high values for any other character studied, whereas S4, with the most significant value for germination has also high but not the highest values regarding IAA, phosphorus and siderophore test. As regards the characters having a direct impact on the development of the plant, it can be observed that E3 has the highest capacity in the mobilization of phosphorous and E2 in the siderophore production. Looking at the IAA production, E3 and M1 showed the highest production. Although IAA is related with plant development and stress tolerance, high amounts of this phytohormone can be phytotoxic. In the case of P2, the strain shows important biocontrol capacities, which can allow the plant to better cope with stress related to fungal infections. In the surfactins assay, strains E4 and P2 showed the highest activity. This activity is related with the capacity of colonizing plants. These two strains showed at the same time the highest capacity in the control of *R. solani*, especially P2, which overgrew and colonized the fungus aggressively, stopping its growth. As regards *S. sclorotiorum*, strain S7 was the most active in the control of the fungus.

To give a résumé, several endophytic bacteria strains isolated from soybean seeds have demonstrated high potential in the improvement of the germination capacity under cold temperatures. Also some bacteria strains have shown ability to be used as biocontrol agents against plant pathogenic fungus. Validation of the findings in a field test will be carried out as next step.

Keywords

16S rRNA · beneficial microorganisms · characterization assays · endophytic bacteria · *Glycine max*

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Table 1: Result of the characterization assays on endophytic bacteria strains isolated from seeds of five varieties of soybean: germination assay (Germ%), phosphorus mobilization assay (P), Siderophores production (sideroph), production of indol-3-acetic acid (IAA), biocontrol assays (*R. solani* and *S. sclerotio*rum) and production of surfactin (Surf). Highest responses are printed in bold.

Strain	Species	Germ% [1]	P	Sideroph (%)	IAA (µg/ml)	*R. solani*	*S. sclerotiorum*	Surf
A1	*Paenibacillus* sp.	ns	+	-	0.85	-	-	-
A2	*Bacillus megaterium*	*	+++	25.00	2.77	+	-	-
A3	*Brevibacillus* sp.	ns	++	32.05	0.34	-	-	-
A4	*Bacillus megaterium*	**	+++	40.06	1.03	-	-	-
E1	*Bacillus megaterium*	ns	++	25.41	0.52	-	-	-
E2	*Paenibacillus* sp.	ns	+++	**77.24**	3.11	-	-	-
E3	*Bacillus megaterium*	*	**++++**	32.05	**34.33**	-	-	-
E4	*Bacillus pumilis*	ns	-	12.15	0.18	**+++**	-	**++**
E5	*Paenibacillus* sp.	ns	-	11.54	1.23	-	-	-
M1	*Bacillus pumilis*	ns	+	11.60	**24.16**	+	-	+
M2	*Paenibacillus* sp.	ns	-	11.19	1.11	-	-	-
P1	*Bacillus pumilis*	***	-	11.60	-	++	+	+
P2	*Bacillus pumilis*	ns	-	14.09	-	**++++**	+	**++**
P3	*Bacillus megaterium*	ns	+++	14.36	1.34	-	-	-
P4	*Paenibacillus* sp.	ns	-	34.94	0.66	-	-	-
S1	*Bacillus megaterium*	ns	+++	29.49	2.39	-	-	-
S2	*Bacillus megaterium*	**	+++	32.91	9.04	-	-	-
S3	*Bacillus subtilis*	**	+++	19.34	NS	+	+	+
S4	*Bacillus megaterium*	***	++	35.47	2.55	-	-	-
S5	*Paenibacillus* sp.	ns	+	8.81	-	-	-	+
S6	*Bacillus megaterium*	ns	+++	28.10	-	-	-	-
S7	*Paenibacillus* sp.	ns	-	-	-	+	++	-

[1] Germ%: significance of the percentage of germinated soyben seeds at 12ºC after 21 days; ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001

P: +, small and diffuse halo; ++++, clear and defined halo; -, no effects observed

Sideroph: percentage of mobilized iron compared to the control positive strain (100%); -, no effects observed

R. solani and *S. sclerotiorum*: +, stopping the fungus growth in the contact zone; ++, stopping the fungus growth without being in contact (halo); +++, bacteria overgrow fungus; ++++, bacteria overgrow the fungus totally; -, no effects observed

Surf: +, small halo; ++, big halo; -, no effects observed

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Genotype dependent microbiome of 60 different tomato cultivars

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Abstract

It is already known that plants influence and shape the bacterial community in their rhizosphere via root exudates. Microorganisms get attracted into the rhizosphere. Some microorganisms can be beneficial to plants by mobilization of nutrients or by preventing the establishment of pathogens. Beneficial microorganism can also play a role in early plant defense by priming the plant. Microorganisms are important players regarding plant health and yield. Some bacteria are already in use as biocontrol agents like *Bacillus thuringiensis*. Still, plant-microbe interactions in the rhizosphere are poorly understood. Understanding the beneficial interaction between bacteria and plant opens the possibility for engineering the microbiome to improve plant performance and plant defense. By analyzing the rhizosphere of 60 tomato cultivars grown under organic conditions the genotype depended microbiome was studied. The goal of the experiment was to understand whether cultivars shape their microbiome differently depending on variety release, fruit type and breeding background.

The experiment was carried out at the Reinshof Experimental Station of the University of Göttingen between May and October 2015. Sixty tomato cultivars were grown in organic low-input conditions in a rainout shelter using a randomized complete block design with eight replications and one plant per plot. This growing system was necessary to exclude the most common pathogens (*Phytophthora infestans* outdoors and *Cladosporium fulvum* indoors) from the trial. The oldest cultivar was released in 1880, the youngest in 2015. The tomato cultivars originate from different breeding backgrounds, they were either bred for conventional or organic production systems; for some cultivars the breeding background remained unknown. The tomato cultivars were also distinguished between salad- and cocktail fruit type. Sampling was done at the end of the season mid-October; all plants were healthy. The plants were pulled up and roots of medium size with adherent soil were gathered from a depth of about 15 cm. Samples were stored at 8°C and later at -60°C. For the metagenomic approach, bacterial DNA was isolated from rhizosphere soil from three plants per genotype using the soil DNA extraction kit (Mobio). For the characterization of the microbiome the gene 16S rRNA was partially amplified using primer 319f and 806r. After the 16S rRNA amplification a second PCR for every sample was performed using Index 1 (N701–N712) and Index 2 Primers (S517–S508) from the Nextera XT Index kit (Illumina, San Diego, CA, USA). The sequence library was prepared using protocols established at AIT and Illumina standard protocols. Two Miseq runs were used to sequence all samples. The obtained fragments were cleaned and with the in house developed pipeline the analysis was performed. For the identification of the bacteria present in the rhizosphere the arbSilva database was used.

This present study shows that the genotype of the tomato cultivars influences the microbiome to some extent. The most abundant operative taxonomic units (OUT) were found in all 60 genotypes. The most abundant phylum with 30% of all identified bacteria species belonged to *Proteobacteria*, which are common in rhizosphere samples all over the world. At the genus level uncharacterized bacteria as well as *Flavobacterium* and *Arthrobacter* have been the most abundant. In contrast to the most abundant taxa less abundant OTUs are genotype specific. The analysis of cocktail and salad showed significant differences between the two groups in the alpha and beta diversity. Several taxa were found to be more abundant in one of the groups. Among them are bacteria like *Tumebacillus* and *Marinicella* which are more abundant in the cocktail cultivars whereas *Nocardioides* and *Mycobacterium* are significantly more abundant in the salad cultivars.

No significant relation between the date of the variety release and the total number of OTUs was found. There is evidence, how-ever, that some modern cultivars count a higher number of OTUs compared to those released between 1880 and 1950. But because less cultivars from this time were in the data set the output maybe biased. The data will be further analyzed if the type of seed production (open pollinator or F1-hybrid) has an influence on the microbial diversity. Additionally the analysis of cocktail and salad tomato will be done separately for the release data and the breeding background. Because the salad and cocktail tomato already show a significantly different structure in the metagenomic structure in the rhizosphere, this may influences the outcome of the more detailed analysis.

Metagenomic approaches put us a big step ahead in identifying the big bacterial players in the rhizosphere. Further research is needed to better understand and use the interactions between bacteria, soil and plant roots

Hönigsberger B, Antonielli L, Samad A, Horneburg B, Trognitz F (2017) Genotype dependent microbiome of 60 different tomato cultivars. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 67. Jahrestagung 2016, 21-23 November, Raumberg-Gumpenstein, pp 57-58. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-45-9

Keywords

16S rRNA · *Lycopersicon esculentum* · microbiota · organic production · rhizosphere

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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Grain yield and quality of winter wheat in Germany: 32 years of progress in official variety trials and on-farm, environmental variability and correlations

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Abstract

After World War II, and after returning to self-sufficiency, still two million tons of high quality wheat had to be imported to Germany to cover the domestic demand because of deficient quality. Already in the early 1970s wheat was produced in sufficient quantity and quality due to new improved varieties with better baking quality. In 2014, the wheat growing area has reached 27% of total arable land. Today, wheat is the most important field crop in Germany. About 51% of the national grain production is used for feeding and 33% for human nutrition, i.e., mostly for milling and baking. Today more wheat is produced than consumed in Germany. Self-sufficiency reached 130% in 2014. How successful was wheat breeding and variety testing in providing improved genotypes and how well was progress transferred into farm production during the last 32 years? The great challenge in wheat breeding towards higher yield and improved baking quality is the strong negative, genetically controlled relation between yield and protein concentration at the one side and the strong positive relation between protein concentration and baking-quality at the other side.

We analysed grain yield, protein concentration, protein yield, falling number, Zeleny sedimentation value and loaf volume from 316 registered varieties tested for their value for cultivation and use (VCU). On-farm data for grain yield, protein concentration, sedimentation value and calculated loaf volume were available from 115 varieties. We applied linear mixed models to estimate gains and losses derived from overall trends (comprising genetic and non-genetic influences), variance components, adjusted variety means and phenotypic correlation coefficients. In Germany, released varieties are graded into five groups according to their baking quality: E (elite wheat - prime quality), A (quality wheat), B (bread wheat) and C (not useable for baking). In VCU trials, A- and B-varieties formed the largest groups (35% and 36%, respectively). On-farm a major shift to varieties with higher quality was observed during the last 32 years. The growing area of A-varieties increased to more than 40%, whereas that of B-varieties dropped to less than 20%. The area of high (E) and the low (C) quality groups covered less than 10%, respectively.

For VCU trials, we found a large significant gain in grain yield (23.8%) , a moderate for protein yield (13.7%), but a significant moderate decline in protein concentration (−8.0%) and loaf volume (−8.5%) relative to 1983 trait levels obtained from overall linear regression. Non-significant gains were obtained for falling number (5.8%) and sedimentation value (7.9%) relative to 1983 (Table 1). On-farm gain in grain yield was 31.6%, but at a mean level about 25 dt ha^{-1} lower than in VCU trials. Improvement of on-farm quality exceeded trial results considerably (Table 1). A shift to varieties with improved baking quality, improved variety-specific nitrogen fertilization and crop management can be considered as main reasons for this remarkable on-farm improvement. Estimates of variance components of traits expressed as per cent of total variation showed that in VCU trials genotypes are of minor influence for protein yield (3%), grain yield (9%), of medium for protein concentration (21%) and falling number (27%), and of major for loaf volume (61%) and sedimentation value (69%). Interaction of genotypes with years and locations are small, but varieties interact more with years than with locations. For falling number, main effects of years (20%) are more important than locations (4%). But for grain and protein yield, and protein concentration main effects of locations have more influence on variation than years. Calculation of correlation coefficients based on adjusted variety means showed that grain yield is strongly negative correlated with protein concentration (*r*= -0.77), moderately with sedimentation value and loaf volume (both *r*= -0.42), but weakly correlated with falling number(*r*= -0.08). Protein yield is associated with grain yield (*r*=0.70), but not so with other traits. Further, our data confirmed the strong relation between protein concentration and sedimentation value (*r*=0.77) and loaf volume (*r*=0.77). On-farm adjusted variety means are stronger related than in VCU trials.

Our study provided evidence that yield progress in VCU trials was successfully transformed to farm yields during the last 32 years, and further that national on-farm wheat quality was considerably improved.

Keywords

Baking quality · breeding progress · environmental variation · correlation · *Triticum aestivum*

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Table 1: Gains and losses for grain yield and quality traits in VCU trials and on-farm. (Overall regression coefficients; percent values are based on 1983 regression estimates; on-farm data for grain yield were obtained from national survey as annual averages; on-farm data for protein concentration, sedimentation value and calculated loaf volume were obtained from national statutory harvest survey as variety×year means).

Traits	Source	Regression estimates				Overall trend - Slope	
		1983	2014	Diff.	%Diff	Absolute	%
Grain yield (dt ha^{-1})	Trial	86.0	106.4	20.4^{***}	23.8	0.659^{***}	0.77
	Trial[1]	85.5	105.6	20.1^{***}	23.5	0.647^{***}	0.76
	On-farm[2]	60.9	80.1	19.2^{***}	31.6	0.620^{***}	1.02
Protein concentration (%)	Trial	13.6	12.5	$- 1.1^{***}$	-8.0	-0.035^{***}	-0.26
	Trial[1]	13.7	12.7	$- 1.0^{***}$	-7.4	-0.033^{***}	-0.24
	On-farm[1]	12.9	13.1	0.2*	1.5	0.008^{*}	0.05
Protein yield (dt ha^{-1})	Trial	11.7	13.3	1.6^{***}	13.7	0.052^{***}	0.44
Falling number (s)	Trial	296.4	313.7	17.3	5.8	0.557	0.19
Sedimentation value (ml)	Trial	40.6	43.8	3.2	7.9	0.103	0.26
	Trial[1]	43.0	47.0	4.0	9.3	0.129	0.30
	On-farm[1]	35.6	51.7	16.1^{***}	45.4	0.542^{***}	1.46
Loaf volume (ml)	Trial	660.8	604.3	-56.5^{***}	-8.5	-1.822^{***}	-0.28
	Trial[1]	667.7	621.1	-46.6^{***}	-7.0	-1.502^{***}	-0.23
Calculated loaf volume (ml)	On-farm[1]	642.4	695.8	53.4^{***}	8.3	1.810^{***}	0.27

Significance levels: *, P≤0.05; **, P≤0.01; ***, P≤0.001; [1] only quality groups E, A and B; [2] all quality groups

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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The dominant dwarfing gene *Ddw1*: a breeder's option to improve lodging resistance in rye

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Abstract

In winter rye (*Secale cereale* L.), the release of the first hybrid varieties in 1984 resulted in significant progress concerning the genetic improvement of agronomic and quality traits as well as biotic stress tolerances. The existence of two heterotic gene pools enabled to exploit heterosis in rye, which resulted in remarkable genetic gains in grain yield. The genetic improvement of lodging tolerance counts among the major breeding goals in rye to further increase grain yield in changing environments. A successful approach to overcome lodging is a reduction of plant height by exploiting dwarfing mutants. In wheat, as well as in rice, the alteration of plant height by dwarfing genes resulted in an increased harvest index (HI). The lesser growth of vegetative organs allowed for a differential partitioning of dry matter from non-reproductive to reproductive organs and, as a consequence, to a significant increase of grain yield in both species. Although recessive dwarfing genes allowed to reduce plant height in rye as well, current hybrid rye varieties do not yet reach a HI comparable to that of wheat. The dominant dwarfing gene *Ddw*1 provides an original opportunity to genetically reduce plant height in rye. *Ddw1* originates from the germplasm collection preserved at the Vavilov Institute of Plant Industry in St. Petersburg and belongs to the group of gibberellic acid (GA)-sensitive dwarfing genes. We have evaluated the testcross performance of genetically diverse semidwarf rye hybrids carrying *Ddw1* in multi-environmental field trials and established a high resolution map of the *Ddw1* locus for the molecular characterization of this gene.

We have developed $F_{4:5}$ near-isogenic inbred lines (NILs), which are homozygous for the recessive (tall) or dominant (dwarf) allele at the *Ddw1* locus, from a cross of a *Ddw1* donor with a tall elite inbred line. The advanced NILs, thus, have a similar genetic background, but differ in the genomic segment carrying *Ddw1*. Experimental hybrids have been established by crossing the NILs to 4 male sterile tester genotypes, which carried either the Gülzower or the Pampa cytoplasm. Field experiments were conducted in 2015 and 2016 in 18 environments (11 locations × two year combinations). Entries were grown on drilled plots of 5 to 6 m² size. Semidwarf and tall hybrids were studied in two separated trials. The experimental design of each experiment was a randomized complete block design (alpha design) with three replications. Next to grain yield (GYD, dt ha^{-1}), all plots have been assessed for heading date (HDT, 1-9, 1 = very late, 9 = very early), thousand grain weight (TGW, g), lodging (LDG, 1-9, 1 = no lodging, 9 = strong lodging) and plant height (PHT, cm). All trials were conducted according to the intensity level 1 defined for value tests performed by the German Federal Plant Variety Office, *i.e*. without application of growth regulators and fungicide treatments.

Statistical analyses were based on plot data of 24 semidwarf as well as 24 tall testcross progenies, the latter of which included 8 released hybrid varieties as checks, which were analyzed separately. All statistical computations were performed with the *R* software package in a two-step procedure. Analyses of variance were performed for all traits in each environment separately. The adjusted entry means from each location were used in a second step to estimate variance components based on the following linear model: $y = G + E + G \times E$, where *G* and *E* denote genotype and environment, respectively. Both factors were treated as random effects. Broad sense heritability (h^2) on an entry-mean basis was estimated from the variance components as the ratio of genotypic to phenotypic variance.

In addition to the phenotypic characterization we have approached *Ddw1* by a previously described comparative genetic approach, which was complemented by a cost efficient RNA-Seq method designated MACE (Massive Analysis of cDNA Ends).

All traits showed significant genotypic and genotype by environment interaction variances. The estimates of broad-sense heritability ranged from $h^2 = 0.82$ for LDG to $h^2 = 0.98$ for PHT. None of the traits deviates from a normal distribution. On average, plant height in hybrids carrying *Ddw1* was 36 cm lower compared to tall hybrids. Likewise, lodging resistance was signifcantly improved in semidwarf hybrids. These results indicate that *Ddw1* enables a cultivation of rye without the application of plant growth regulators. The substantially improved lodging tolerance of semidwarf

Hackauf B, Musmann D, Braun E-M, Krezdorn N, Rotter B, Winter P, Fromme FJ, Melz G (2017) The dominant dwarfing gene *Ddw1*: a breeder's option to improve lodging resistance in rye. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 67. Jahrestagung 2016, 21-23 November, Raumberg-Gumpenstein, pp 61-62. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-45-9

hybrids contributes to increase rye productivity by raising the speed of the harvest, higher grain quality and reduced drying costs.

The trials were affected by drought stress conditions in both years. Interestingly, semidwarf hybrids significantly outperformed their near isogenic tall control entries with an increase of 16% in grain yield for the best performing semidwarf genotypes. It is noteworthy, that grain yield in the tall entries is weakly ($r = 0.19$), albeit significant ($p<0.05$), correlated with lodging tolerance. However, the observed yield advantage of semidwarf hybrid varieties under low cultivation intensities demonstrates, that *Ddw1* can boost the use of rye for low input farming systems and supports a sustainable production of rye for food and feed.

It is becoming increasingly evident that the GA class of plant hormones is of pivotal relevance in the response of plants to abiotic stress. The combination of improved lodging tolerance and increased yield under natural occuring drought conditions, that we observed in semidwarf rye hybrids with altered GA content, identifies the use of mutants affecting the GA biosynthesis pathway as a viable option particularly for hybrid breeding programs to create lodging tolerant and climate-resilient crops.

The comprehensive genetic analysis in segregating progenies originating from the cross R1620 (tall) × R347/1 (dwarf) confirmed the monogenic dominant inheritance of *Ddw1*. Next generation sequencing based transcript profiling revealed novel insights in gene expression patterns and allele frequencies of semidwarf compared to near isogenic tall inbred lines. The developed molecular markers enable the introgression of *Ddw1* in elite rye germplasm with unprecedented precision and accelerate the progress in breeding of semidwarf rye hybrids.

Keywords

Drought tolerance · gibberelic acid · hybrid breeding · SMART breeding · *Secale cereale*

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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AmyCtrl – Genome-based prediction for pre-harvest sprouting tolerance in wheat

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Abstract

Pre-harvest sprouting (PHS) describes the germination of mature seeds before harvest. During the germination process, α-amylases degrade the starch which leads to reduced falling numbers, an important economic trait concerning the dough quality of wheat. However, the extent of PHS is highly dependent on the annual weather and without PHS inducing conditions, a differential response of wheat cultivars for falling number cannot be assessed. The ongoing project “AmyCtrl” focuses on the validation of molecular markers for controlling untimely α-amylase activity and the selection for PHS tolerance in breeding material derived from Austrian and German wheat breeders. These molecular markers were identified in recent projects to be associated with falling number, falling number stability, and PHS tolerance. Furthermore, genomic selection methods will be implemented for the evaluation of PHS tolerance in unselected material.

The material included 400 breeding lines which were derived from five Austrian and German breeding companies and represents F_6 lines not selected for PHS tolerance. The calibration set comprised 200 lines analyzed in the seasons 2014/2015 and 2015/2016 and a validation set of 100 lines was exchanged across seasons. The material showed a narrow genetic relatedness while the breeding material from Austria was less related to the German breeding material. In both seasons, field trials were conducted in five German and Austrian locations with an unreplicated augmented design using checks to adjust for block effects. The traits germination index, sprouting of intact ears and falling number have been assessed for each location. In addition to the standard procedures, a test has been developed to determine falling number stability under controlled conditions. For this, the kernels are stored at room temperature (after-ripening) until wetting to induce a decrease of falling numbers and a better differentiation of falling numbers between lines. For genomic selection, different models including a random polygenic effect for the breeding lines, i.e. genome-based best linear unbiased prediction (GBLUP) models, were applied to the data from season 2014/2015. In addition, molecular markers linked to major quantitative trait loci (QTL) were included as a fixed effect in another prediction model. These QTL included *Rht-B1* and *Rht-D1* for falling number and *Phs1* for

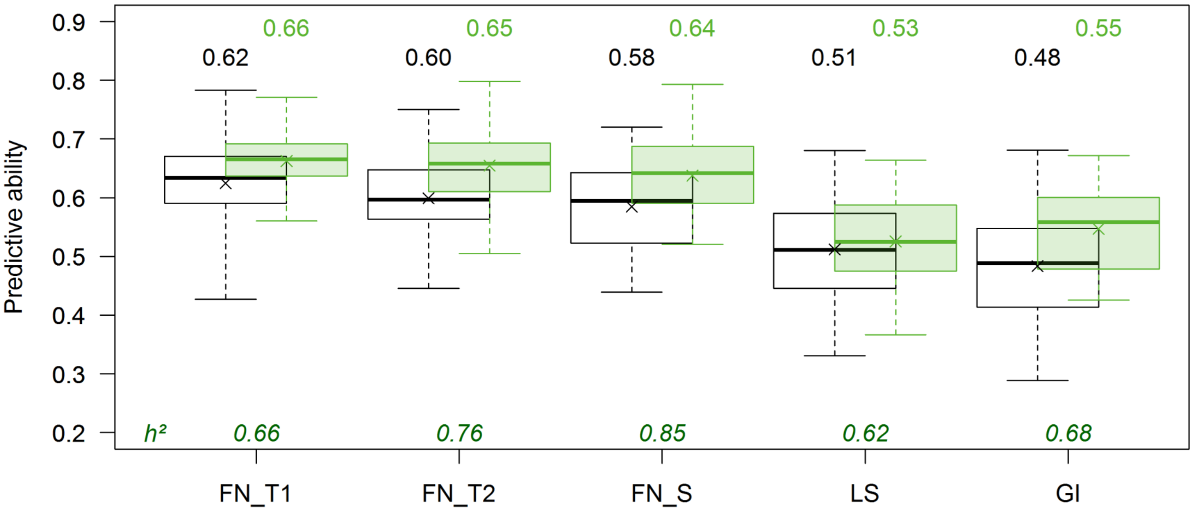

Figure 1: Boxplots of predictive abilities from 50 cross-validation runs for the traits falling number at harvest (FN_T1), falling number at delayed harvest (FN_T2), falling number stability (FN_S), sprouting of intact ears (LS), and germination index (GI). Left boxplot for each trait represents simple GBLUP and right boxplot the GBLUP model with accounting for major QTL. Numbers above the boxplots indicate average predictive abilities, numbers below boxplots indicate heritabilities for each trait.

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PHS-associated traits. Predictive ability of the models was assessed with five-fold cross-validation replicated ten times to result in 50 different estimation and test sets. While the model is calibrated on the estimation set including 4/5 of the data from season 2014/2015, the predictive ability is derived from the correlation between predicted and observed breeding values in the test set comprising 1/5 of the data set.

Average predictive abilities for the season 2014/2015 varied from 0.48 to 0.62 for the PHS traits and falling number with the GBLUP model (Figure 1). Fitting markers linked to the major QTL in the GBLUP model increased predictive abilities for all traits. Highest predictive abilities were observed for falling number, but might be overestimated within one growing season. Overall for traits like falling number and PHS tolerance, major QTL play an important role and accounting for these QTL in the prediction model increase predictive abilities. However, the results are a first indication for predicting PHS tolerance in unselected wheat breeding material and the observed predictive abilities need to be validated across seasons.

Keywords

α-Amylase activity · dormancy · dwarfing genes · falling number stability · marker-assisted selection · *Triticum aestivum*

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Screening of wheat lines for fertility restoration of cms lines based on *T. timopheevii*

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Abstract

The RESTORER project, set up by six German plant breeding companies together with the Bavarian State Research Center of Agriculture (LfL) and the Leibniz-Institute for Plant Genetics and Crop Plant Research (IPK) intends to establish a cytoplasmic male sterility (cms) system for an efficient breeding of hybrid wheat (*Triticum aestivum*). The cms system based on alloplasmic maternal lines with *T. timopheevii* cytoplasm promises environmentally stable pollen sterility and high receptiveness to cross-fertilisation. While defective mitochondrial genes of the *T. timopheevii* cytoplasm induce cms, nuclear male fertility restoration genes (*Rf*) are able to compensate this loss of functional genetic information. However, none of the *Rf* genes described so far for wheat is able to ensure full fertility restoration. Therefore, the ultimate objective of the two work packages (WP) in RESTORER is the development of effective molecular selection markers to accumulate dominant *Rf* genes in cms hybrid wheat. WP1, coordinated by the LfL and described below, is focused on the identification, characterization and genetic mapping of wheat *Rf* genes. The other part of the project, organized by the IPK, aims at the molecular characterization of the pentatricopeptide gene family in wheat as potential source of *Rf* genes.

WP1 involves test crosses in the greenhouse between cms line AoSperber and different paternal lines. These include adapted winter and spring wheat varieties, synthetic wheats, exotic genotypes, wheat-*T. timopheevii* introgression lines, and wheat-alien translocation lines. After the hand crosses of the paternal lines with the cms line AoSperber in a greenhouse pre-test, the F_1 generation was grown up and self-pollinated. The restoration capacity (RC) of the self-pollinated F_1, F_2 and BC_1 plants was determined by dividing the number of grains by the number of spikelets for the four main ears of a plant. All paternal lines showing RC were tested with a recently developed molecular selection marker for the *Rf3* gene. In order to eliminate environmental effects, field trials will be conducted with restoring F_1 hybrids at six locations. F_1 hybrids with RC >1.0, indicating the presence of one or more *Rf* genes, will be used for multi-location field trials irrespective of the presence of *Rf3*. F_1 hybrids with RC 0.4-1.0 will be only included when the *Rf3* gene is absent and F_1 hybrids with a RC <0.4 are discarded. For the field trials, a relative restoration capacity (RC%) will be calculated by dividing the RC of a special F_1 hybrid by the RC of a F_1 hybrid of the restorer reference line Primepi. The num-

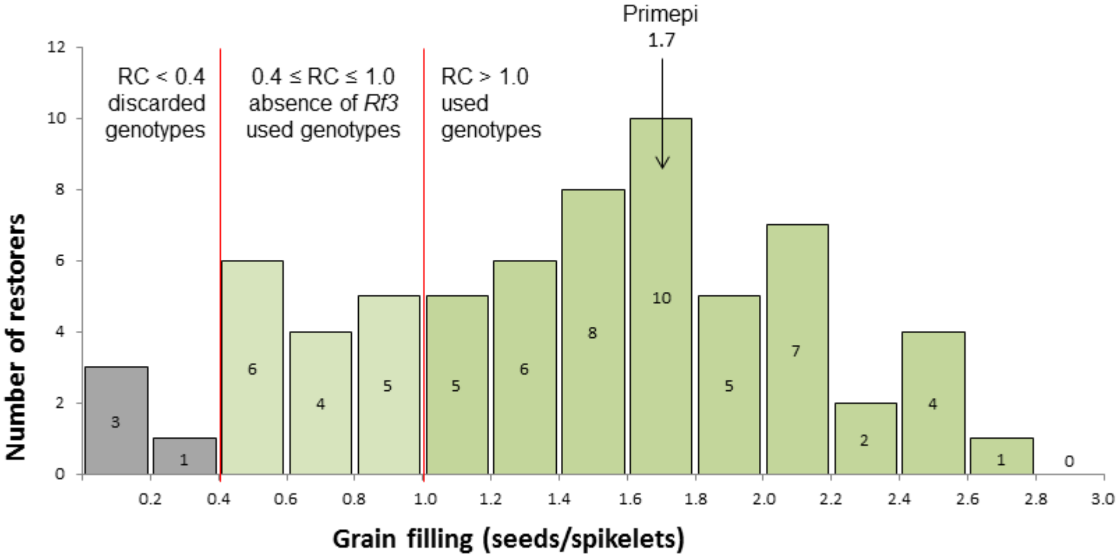

Figure 1: Histogram of the tested F_1 genotypes with a bar for each grain filling class. The limits of a grain filling class with a width of 0.2 seeds/spikelet are shown. The decision limits for genotypes to be tested in multilocation field trials are symbolized by two vertical lines. Genotypes with a restoration capacity (RC) lower than 0.4 were discarded, genotypes with a RC over 1.0 are being used and genotypes with an RC between 0.4 and 1.0 are being used only when the *Rf3* gene is absent.

Block A, Mohler V, Schmidt S, Bund A, Hartl L (2017) Screening of wheat lines for fertility restoration of cms lines based on *T. timopheevii*. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 67. Jahrestagung 2016, 21-23 November, Raumberg-Gumpenstein, pp 65-66. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-45-9

ber and effect of the involved *Rf* genes will be investigated in segregating mapping populations.

The test crosses of 2015 and 2016 were completed and those of 2017 are almost already planned, resulting in a current sum of 1637. For 2015 the results are presented. Of 577 test crosses, 409 F_1 hybrids were successfully grown up and self-pollinated, resulting in 67 restoring genotypes (16% of 409 F_1), from which 18 paternal lines were *Rf3* positive (16% of 409 F_1). In cropping season 2017, multi-location field trials will be performed for 55 F_1 hybrids: Of these, 48 showed RC >1.0 in the greenhouse, whereas for seven with RC between 0.4 and 1.0 *Rf3* was absent. Twenty-seven segregating mapping populations are being built up, consisting of 20 BC_1 and 7 F_2 populations.

Keywords

Cytoplasmic male sterility · genetic mapping · hybrid wheat · *Triticum aestivum*

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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Optimum breeding strategies in hybrid cereal breeding programs

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Abstract

Each breeding program is characterized by a specific combination of the biological and reproductive features of the crop, budget, costs, variance components and technology available. Currently, each breeder has to decide if, how, and when to implement new technologies as genomic selection according to its specific resources and objectives.

We performed model calculations to compare the advantage of five breeding strategies upon variations on available budget, costs for line and hybrid seed production as well as variance components for grain yield. Additionally, we include a stage of per se preselection in nurseries for disease resistance before genomic or phenotypic selection on hybrid grain yield.

One breeding strategy, *GSrapid*, with moderate nursery selection followed by genomic selection and one stage phenotypic selection maximized annual selection gain for grain yield across a wide range of budget, cost and variance component scenarios. This was consistent for maize, wheat, rice, rye, barley and triticale, highlighting the importance of genomic selection in hybrid cereal breeding. Implementing nursery preselection caused reductions in the selection gain of grain yield, especially if selected fractions were smaller than 0.25 and genomic prediction accuracy was larger than 0.3.

The succesful use of genomic selection requiered an increased number of test candidates entering the breeding strategy. In our calculations, we found a larger impact on the annual selection gain for the costs for DH line production compared to the cost of hybrid seed production. On this basis, current research efforts to improve on DH line production, by enhancing haploid induction rate assessment, chromosome doubling techniques and phenotypic markers for the identification of haploid seeds are of major importance to exploit genomic selection in cereal breeding. For hybrid breeding programs with low budget, high hybrid seed production costs and large proportion of masking variances relative to genetic variance, as the case of hybrid triticale, we found a large advantage for *GSrapid* over traditional breeding strategies. This result unravel the expected benefit of implementing genomic selection not only in high budget but also in low budget breeding programs.

Keywords

Genomic selection · grain yield · model calculations · multistage testing · preselection in nurseries

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Genomic assisted selection for enhancing line breeding: merging genomic and phenotypic selection in winter wheat breeding programs with preliminary yield trials

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Abstract

The selection of lines that enter resource-demanding multi-environment trials is a crucial decision in every line breeding program as a large amount of resources are allocated for thoroughly testing these potential varietal candidates. We compared conventional phenotypic selection with various genomic selection approaches across multiple years as well as the merit of integrating phenotypic information from preliminary yield trials into the genomic selection framework. The prediction accuracy using only phenotypic data was rather low ($r = 0.21$) for grain yield but could be improved by modelling genetic relationships in unreplicated preliminary yield trials ($r = 0.33$). Genomic selection models were nevertheless found to be superior to conventional phenotypic selection for predicting grain yield performance of lines across years ($r = 0.39$). We subsequently simplified the problem of predicting untested lines in untested years to predicting tested lines in untested years by combining breeding values from preliminary yield trials and predictions from genomic selection models by an heritability index. This genomic assisted selection led to a 20% increase in prediction accuracy, which could be further enhanced by an appropriate marker selection for both grain yield ($r = 0.48$) and protein content ($r = 0.63$). The easy to implement and robust genomic assisted selection gave thus a higher prediction accuracy than either conventional phenotypic or genomic selection alone. The proposed method took the complex inheritance of both low and high heritable traits into account and appears capable to support breeders in their selection decisions in order to develop enhanced varieties more efficiently.

Keywords

Baking quality · dough rheology · genomic prediction · genomic selection · *Triticum aestivum*

Acknowledgements

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67. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs
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Agronomic performance of hooded and awnless spring barley mutants

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Abstract

Barley is one of the most important feed crops, with an acreage of about 50 Mio. ha worldwide. Barley is mainly used as grain for feed and malting, but delivers also high yields if used as pasture, whole-plant silage or hay. For whole-plant use, ‘awnless’ types are preferred as the long awns of barley cause injuries in the muzzles of livestock. The dominant *Kap1* mutation on chromosome 4HS leads to the formation of a so-called *hood*, which is an inverted infertile additional floret at the top of the outer lemma instead of awns. *Lks* mutants cause a reduction of awn length in case of *Lks2* and *Lks5*, and to complet lack of awns in case of *Lks1*.

Within a project coordinated by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture a wide range of barley mutant genetic stocks were tested from 2015-2016 to evaluate their agronomic performance compared to some check varieties.

The mutant lines were evaluated for growth development, plant height and ground coverage at different growth stages, leaf diseases (*i.e*. net blotch, powdery mildew, leaf rust), grain, straw and total biomass yield and grain characteristics (*i.e*. thousand grain mass, seed plumpness >2.5 mm).

The majority of the tested germplasm was inferior in almost all traits compared to the tested check varieties. However, a few mutant genetic stocks are promising with respect to some key traits, e.g. biomass yield and grain yield, leaf disease resistance, juvenile growth development. For example, within each mutant group a few genotypes were identified with similar or even higher biomass yield compared to the check varieties ‘Eunova’, ‘Alpina’ and ‘Optic’. The majority of genotypes, however, was signficiantly inferior. Biomass was significantly correlated to grain yield (r=0.72***). Thousand grain mass and percentage of plump seeds (>2.5 mm), however, was significantly higher in the wild type (check) germplasm (40.2 g and 79.4%) than in the two awnless (*Kap1*, *Lks1*) groups (29.0-30.6 g; 27.5-29.6%). A major drawback in the mutant genetic stocks was a high susceptibility to lodging in almost all genotypes. In a summary, about five to six hooded and/or awnless genotypes were identified as favourable resources for specific forage barley breeding programmes.

Keywords

Awn · forage · *Hordeum vulgare* · mutant genetic stocks · whole-plant use

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Figure 1: Hooded (*Kap1*) and awnless (*Lks1*) mutants in barley. In the hooded mutant the awn is reduced to an inverted floret (left), in the awnless *Lks1* mutant (right) the awns are reduced to little or no appendages on the lemma, depending on the source of the Lks1 gene and the genetic background. The ectopic expression of the *Kap1.a* allele is associated with a 305 base pair duplication in intron 4 of the *Knox3* sequence. The *Lks1* gene is closely linked to the *vrs1* (six-row type) gene on chromosome 2HL.

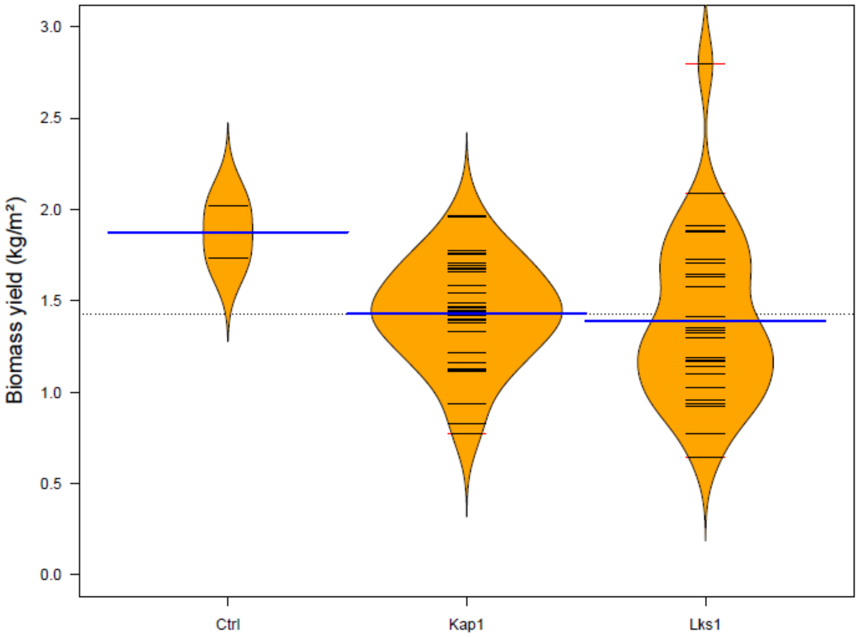

Figure 2: Violin plot displaying the variability of biomass yield of wildtype (Ctrl) check varieties, hooded (*Kap1*) and awnless (*Lks1*) mutant genetic stocks. Data are mean values from field trials performed in 2015 and 2016 in Raasdorf; solid blue lines represent the means in the respective mutant group; awned check varieties (Ctrl) were two-rowed varieties 'Optic' and 'Eunova'.