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Reference genome sequence and its implications for a 'perfect marker' development

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Standard breeding procedures require large scale screening for many generations and selection of desired traits mainly by looking on phenotype. This requires time and significant investment to human labor and space. Marker assisted selection (MAS) with aid of high-throughput genotyping can speed the process by selection of desired lines in very early stages of ontogenetic development which saves time and space. However, availability of markers with tight linkage to desired traits is prerequisite for MAS. The perfect marker for MAS is the marker which never segregates from the followed trait. Such markers can be developed only from genes responsible for variation of the traits and positional cloning is the method to identify the genes. In plant crops with large and complex genomes like wheat, the positional cloning is a long, expensive and laborious process. On the other hand the reference genome can serve as mapping backbone for high-density mapping as was shown on cloning of *QPm-tut.4A* gene introgressed to hexaploid wheat from tetraploid *Triticum militinae* or Targeted chromosome-based cloning via long-range assembly (TACCA) demonstrated on cloning of *Lr22a* gene. The MutRenSeq approach uses the reference sequence as a source of exon information for NBS-LRR exon-capture. So availability of reference genome sequence facilitates the Positional cloning by about ten times and also supports diversity of approaches for gene cloning.

Keywords

High density mapping · leaf rust · marker assisted selection · powdery mildew · *Triticum* · wheat

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Is there a type VI resistance against Fusarium head blight in wheat?

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Fusarium head blight (FHB) is one of the most devastating diseases of wheat. Besides serious reduction of the yield potential, the infection produces misshapen, shriveled grains and contaminates the produce with different types of mycotoxins. Arguably, this aspect is economically the most important aspect as representing a severe threat to food and feed safety. Recent studies revealed also a potential damage to baking quality traits in infected grains. Taken together and considering the technological and economical importance of FHB in wheat, kernel resistance is probably the most important aspect to prevent damage. Yet, kernel resistance is difficult to grasp and rather complicated to phenotype.

Several types and components of resistance to FHB were described. Ear resistance is composed of resistance against the primary infection (type 1) and the resistance against the spreading of the infection throughout the spike (type 2). The ear symptoms are scored after flowering. Several kernel specific resistance types have been identified such as the proportion of healthy-looking grains (type 3), yield stability (type 4) and the resistance against the accumulation of mycotoxins (type 5). Several studies have shown that the degree of the decline of baking quality in FHB infected grains differs in different wheat varieties.

The aim of the present study was to evaluate in which measure the stability of baking quality is linked with other kernel resistance type and if it is about a discrete resistance in certain varieties. For this, we tested 6 wheat varieties with known differences in the kernel resistance in field trials at 3 sites and for 2 years (= 6 environments). The trial was a split-plot design with 6 replicates. Each plot contained all varieties either infected or non-infected (control). After harvest, we measured the grain yield, the thousand kernel weight (TKW), the specific weight (test weight), the proportion of grains damaged by Fusarium, and the content in the mycotoxin deoxynivalenol (DON). Furthermore, some rheological properties of the dough have been tested, *i.e.* water absorption capacity and the kneading resistance.

Overall, in this study, we found only a weak correlation between the reduction of baking quality and the other types of damages on the grains, suggesting that the resistances are independently inherited. We conclude that there is a specific resistance type that can prevent the decay of rheological properties of the dough. This is the additional sixth resistance type present in wheat.

Keywords

Baking quality · *Fusarium graminearum* · kernel resistance · *Triticum aestivum*

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Improving Fusarium head blight resistance in durum wheat

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Fusarium head blight (FHB) is one of the most destructive diseases of durum wheat (*Triticum durum*). FHB resistance breeding in durum is, however, hampered by the limited variation in the elite gene pool and difficulties in efficiently combining the numerous often small-effect resistance QTL in the same breeding line.

We evaluated an international collection of 228 genotyped durum cultivars for FHB resistance over three years to investigate the genetic architecture and potential of genomic-assisted resistance breeding. Moreover, we introgressed resistance alleles from relatives (*i.e.* *T. aestivum*, *T. dicoccoides*, *T. dicoccum*), developed multi-parental populations (750 lines) and phenotyped these pre-breeding lines over two years. Although a lack of highly resistant lines was evident for both collections, broad variation was found, including many moderately resistant pre-breeding lines. Plant height strongly influenced FHB resistance levels and led to co-localization of plant height and resistance QTL. Notwithstanding, a major QTL on chromosome 3B was identified in the elite durum material independent of plant height. Comparison between phenotypic and genomic selection for FHB resistance in the elite germplasm revealed a superior prediction ability of the former, nevertheless simulated selection experiments resulted in higher selection responses when using genomic breeding values for early generation selection. An earlier identification of the most promising lines was furthermore feasible with a genomic selection index, which suggested a much faster short-term population improvement than previously possible in durum. In the long-term, exotic germplasm can broaden the genetic base for FHB resistance beyond the capabilities of elite material for achieving higher levels of resistance.

Keywords

Genome-wide association mapping · genomic selection · plant height · *Triticum durum*

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Gain by loss: High resolution mapping of the Fusarium head blight resistance QTL *Qfhs.ifa-5A* in wheat

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Fusarium head blight (FHB) is a severe fungal disease of wheat affecting yield and grain quality. One major QTL for FHB resistance in wheat is *Qfhs.ifa-5A*. Genetically fine-mapping separated the initial *Qfhs.ifa-5A* interval into two QTL: *Qfhs.ifa-5Ac* (mapped across the centromere) and *Qfhs.ifa-5AS* (mapped on 5AS). Although the genetic intervals of *Qfhs.ifa-5Ac* and *Qfhs.ifa-5AS* were as small as 0.1 and 0.2 cM, the corresponding physical distances were 44.1 Mbp and 49.2 Mbp large, respectively.

To further increase the map resolution a radiation induced deletion mapping approach was performed. Two gamma irradiated wheat deletion panels were created: (i) radiation selfing (RS) panel: seeds of line NIL3 carrying the *Qfhs.ifa-5A* resistance allele in a susceptible background were irradiated and plants thereof were selfed to obtain deletions in homozygous state; and (ii) a radiation hybrid (RH) panel: irradiated pollen of the wheat line Chinese Spring (CS) was used for pollinating the CS-nullisomic-5A-tetrasomic-5B.

More than 5000 RS and 276 RH plants were pre-screened for deletions on 5AS. Plants having one or more markers deleted were analysed using 102 5AS-specific markers. A consensus map derived from both deletion panels resulted in a 380-fold map improvement (cR/cM) for the QTL-interval on the 5AS chromosome com-

pared to the genetic mapping approach, with an average map resolution of 0.65 Mb/cR. This striking improvement in map resolution underlines the superiority of radiation induced deletion mapping over genetic linkage mapping for low recombining regions. Phenotyping the RS deletion lines can help to narrow down the QTL-intervals for gene cloning.

Keywords

Deletion mapping · *Triticum aestivum*

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Vitality of wheat affected by *Fusarium* head blight: physiological reactions

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Bread wheat (*Triticum aestivum* L.) is a staple food in more than 40 countries of the world. The wheat acreage is growing due to its flexible adaptation to a wide range of environments caused by an enormous genetic variation. Bread wheat has its major importance in human and animal nutrition, however, devastating fungal diseases such as *Fusarium* head blight (FHB) can cause significant grain yield losses and end-use quality reduction. Several disease management strategies, e.g. fungicide spraying, crop rotation and residue treatment, are used to decrease and/or eliminate yield losses and mycotoxin production due to FHB infection. The development of resistant cultivars is the most effective way for managing FHB. A positive relationship between non-specific disease resistance and different grain colors determined by bioactive pigments, e.g. carotenoids and anthocyanins, was observed. The objective of this study was to determine the relationship between the sensitivity to FHB of wheat varieties with colored grains and physiological responses during flowering on the flag leaf which is traditionally considered the main photosynthetic organ for grain filling.

The study was carried out from 2016 to 2018 with 25 spring wheat varieties and breeding lines with colored grains. Artificial inoculation of spikes was performed by spraying a suspension of *Fusarium*

culmorum spores at Zadoks growth scale 65. Infected spikes were measured for different physiological characteristics, i.e. rate of photosynthesis, rate of transpiration and stomatal conductance. Further, the infection rate was determined by visual scoring of the symptoms.

Several genotypes of colored grained wheat were detected with good resistance to *F. culmorum*. However, it could have not been proved that the wheat grain color and its responsible pigment is associated with the improved FHB resistance, but that other genetic or chemical factors influence the resistance.

Keywords

Fusarium culmorum · photosynthesis · physiology · stomatal conductance · transpiration · *Triticum aestivum*

Acknowledgments

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The use of wild relatives and chromosome genomics for gene mapping, cloning and improving rust resistance in wheat

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Wild Triticeae species like *Aegilops*, *Agropyron* or *Secale* represent a huge reservoir of useful genes and alleles providing resistance to pests and diseases which would be desirable to transfer into wheat by interspecific hybridization. The most popular method to identify and follow the alien chromatin in wheat genetic background is the *in situ* hybridization of alien genomic- (GISH) and/or repetitive DNA (FISH) probes. However, the weak point is that the throughput of the molecular cytogenetic methods is low.

In the frame of the Martonvásár pre-breeding programme, the leaf rust resistant *Ae. biuncialis* MvGB642 accession was crossed with wheat genotype Mv9kr1. The molecular cytogenetic (GISH and FISH) selection identified two wheat-*Ae. biuncialis* BC₃F₃ genotypes with seedling stage resistance to leaf rust. One contains a 1DL.1DS-U disomic translocation and a 2M chromosome, while the another contains a 5DS.5DL-M disomic translocation. *Agropyron glael*, a hybrid of *A. glaucum* and *A. elongatum*, showed re-

sistance to leaf rust under field conditions. The cytogenetic screening identified a genotype carrying a 6D-*Agropyron* disomic translocation which also showed resistance to leaf rust. The perennial rye cultivar 'Kriszta', a hybrid of *S. cereale* and *S. montanum*, is resistant to leaf rust, stem rust, stripe rust and powdery mildew. The stripe rust resistant BC₂F₉ line '179' was selected under field conditions and the cytogenetic analysis proved the presence of a disomic 1RS.1BL centric fusion, which has different allele compositions from the well-known Petkus derived 1RS arm.

In order to improve the throughput of the introgression breeding process and for map-based cloning of resistance genes, a better knowledge on the genome structure of wild gene source species will be needed. However, the big genome size, the high percent of repetitive sequences and often the polyploid structure hampers the genome analysis of wild Triticeae species.

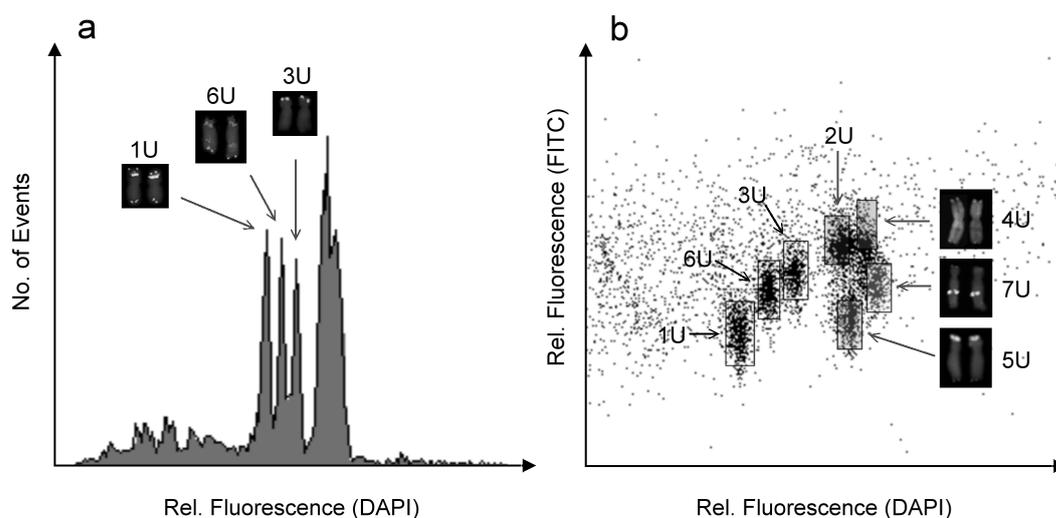


Figure 1: Flow karyotyping and flow sorting of *Aegilops umbellulata* chromosomes: Monoparametric flow karyotype of DAPI-stained mitotic chromosomes (a) comprises three chromosome specific peaks representing chromosomes 1U, 6U and 3U, and a composite peak of the remaining four chromosomes; (b) biparametric dot plot flow karyotype of *Ae. umbellulata* obtained after fluorescent labelling of chromosomes by FISHIS with the probe FITC-(GAA)₇-FITC and by DAPI staining. Double labelling resolves all seven chromosomes of *Ae. umbellulata*.

To overcome the difficulties, the Olomouc team has been developing chromosome-centric approaches, which rely on the ability to dissect nuclear genomes to chromosomes. This is achieved by preparing suspensions of intact mitotic chromosomes from root tip meristems and flow-sort the target chromosomes. Discrimination of individual chromosomes based on DNA (DAPI-) fluorescence alone requires differences in size between chromosomes in a karyotype, a condition which is not frequent. Chromosome discrimination may be improved after fluorescent labelling by *in situ* hybridization in suspension (FISHIS) those DNA repeats, which are distributed unevenly among the chromosomes (Fig. 1). For wild relatives of wheat such as *Aegilops*, *Agropyron* and *Secale*, where reference pseudomolecules are not yet available, chromosome genomics provides a cost-effective way to identify a majority of genic sequences and order them along chromosomes. As demonstrated in wheat, chromosome-derived sequences facilitate the development of DNA markers to support alien introgression breeding. With the growing number of finished reference genome sequences for important Triticeae species, the future of chromosome genomics lies in the ability to target particular genome regions. This results in a significant reduction of cost and, if needed, it allows analyzing a chromosome of interest isolated from different cultivars, landraces and mutants. The applications include characterization of alien chromatin in introgression lines and development of molecular markers. Gene cloning has become one of the most important applications of chromosome genomics, recently. The targeted approach greatly streamlines gene cloning and reduces project costs. Two chromosome-based gene cloning approaches, namely MutChromSeq and TACCA (targeted chromosome-based cloning via long-range assembly) have been developed and validated and are being increasingly used.

Keywords

Aegilops biuncialis · *Agropyron glael* · FISHIS · flow cytometric chromosome sorting · introgression breeding · *Triticum aestivum*

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

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Abstract

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

Keywords

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

Introduction

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

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

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

Material and methods

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

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

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

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

Results and discussion

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

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

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

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

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

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

In this study, lines with multiple resistance genes were not infected by any of the races. However, some combinations of dual resistance are relatively easily overcome by the development of new virulence races of the pathogen, given that virulence against the parent resistance gene are present in pathogenic races in the regi-

on (Hoffman 1982). Therefore, a safer strategy is to combine resistance genes where virulence against at least one of the genes are rare. In Europe, virulence is frequently found against *Bt7* and relatively common also against *Bt1*, *Bt2* and *Bt5*. Our study shows that these genes are also found in several commercial varieties in Europe, and this is likely the reason for the virulence. Using these resistance genes alone can, therefore, not be used as the only strategy to control the disease, but must be combined with other control measures or at least be followed with seed analysis for the presence of spores prior to sowing.

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

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Bt1

PI-554-108	6.9	0.0	0.0	21.7	20.7	0.0	0.0
PG3540	0.0	0.0	0.0	52.9	26.7	0.0	0.0
Starke NIL Bt1	2.4	0.0	0.0	36.5	8.2	0.0	0.0
Butaro	0.0	0.0	0.0	37.1	70.6	0.0	0.0
Courier	0.0	0.0	0.0	48.8	37.5	2.2	0.0
Ring 61: HSI-664-11	0.0	0.0	0.0	29.8	10.9	2.2	0.0
ButQue 1	0.0	0.0	0.0	17.1	51.7	0.0	0.0
ButQue 2	0.0	0.0	0.0	10.0	13.5	0.0	0.0
ButQue 3	0.0	0.0	0.0	19.4	48.9	0.0	0.0
ButQue 4	0.0	0.0	0.0	30.0	43.6	0.0	0.0
ButQue 5	0.0	0.0	0.0	12.6	16.7	0.0	0.0
ArPG	0.0	0.0	0.0	40.0	20.0	0.0	0.0
PerBut 1	0.0	0.0	0.0	38.3	10.0	0.0	0.0
PerBut 2	0.0	0.0	0.0	55.9	58.3	0.0	0.0
PerBut 3	0.0	0.0	0.0	51.4	68.0	0.0	0.0
PerBut 4	0.0	0.0	0.0	38.5	14.6	0.0	0.0
PerKo 1	0.0	0.0	0.0	47.5	17.1	0.0	0.0
PerKo 2	0.0	0.0	0.0	23.7	11.8	0.0	0.0
PerXe 1	0.0	0.0	0.0	68.2	22.6	0.0	0.0
PerXe 2	0.0	0.0	0.0	62.5	47.8	0.0	0.0
PerXe 3	0.0	0.0	0.0	48.8	55.3	2.2	0.0
CoPG 1	0.0	0.0	0.0	26.0	18.4	0.0	0.0
CoPG 2	0.0	0.0	0.0	47.5	17.1	0.0	0.0
CoPG 3	0.0	0.0	0.0	57.7	18.0	0.0	0.0
CoPG 4	0.0	0.0	0.0	47.3	26.3	0.0	0.0
FoPG	0.0	0.0	2.0	12.5	7.4	0.0	0.0
DZW1107p	0.0	0.0	0.0	25.0	22.6	2.4	0.0
AB-5	0.0	0.0	0.0	16.0	22.0	0.0	0.0
CCP 16	0.0	0.0	0.0	22.0	7.8	0.0	0.0
CCP-20	0.0	0.0	0.0	15.1	5.3	2.2	0.0
CCP-29	2.2	0.0	0.0	10.0	13.5	0.0	0.0
CCP-34	0.0	0.0	0.0	9.1	25.6	0.0	0.0
Butaro	0.0	0.0	0.0	38.7	70.0	4.3	2.3
HSI 439-2-10	0.0	0.0	0.0	17.5	55.3	0.0	0.0
HSI 447-10	0.0	0.0	0.0	7.0	16.7	0.0	0.0
HSI 672-10	0.0	0.0	0.0	14.7	10.3	4.8	0.0
HSI 528-11	0.0	0.0	0.0	11.1	2.4	0.0	0.0
HSI 664-11	0.0	0.0	0.0	9.1	8.2	2.2	2.6
HSI 612-11	0.0	0.0	0.0	11.4	14.7	0.0	0.0
HS 191-04	0.0	0.0	0.0	31.9	30.8	0.0	0.0
WIF109Bl.01	0.0	0.0	0.0	44.0	42.2	0.0	0.0
WIF9Bl.741	0.0	0.0	0.0	32.3	81.9	0.0	0.0
WAR109Bl.01	0.0	0.0	0.0	23.8	12.0	2.4	0.0
SePen 6A	0.0	0.0	15.2	37.5	25.0	47.1	4.2
DZW1107c	0.0	0.0	2.2	26.3	7.7	43.6	0.0
DZW1107h	0.0	0.0	0.0	21.4	41.3	10.8	0.0
DZW1107i	0.0	0.0	0.0	25.9	19.0	6.5	0.0
DZW1107k	0.0	0.0	0.0	28.6	22.0	17.2	2.6
DZW1107m	0.0	0.0	0.0	19.4	20.0	23.3	2.9
DZW1108a	0.0	0.0	0.0	6.3	20.0	18.0	0.0
DZW1108b	0.0	0.0	0.0	14.8	46.2	25.5	0.0
DZW1019a1	0.0	0.0	0.0	6.3	5.7	20.4	0.0
DZW1019e	0.0	0.0	0.0	8.9	0.0	10.2	0.0
DZW1019h1	0.0	0.0	0.0	4.3	3.8	8.0	0.0
DZW1019h2	0.0	0.0	0.0	6.3	22.0	12.2	0.0
DZW1019p1	0.0	0.0	0.0	6.3	8.7	20.0	0.0
DZW1019g	0.0	0.0	0.0	5.7	5.4	5.4	0.0
HSI 367-10	0.0	0.0	0.0	39.7	16.7	21.6	0.0

BtZ

DZW1109b	0.0	0.0	0.0	0.0	0.0	44.4	0.0
DZW1110	0.0	0.0	0.0	2.2	0.0	79.5	0.0
DZW1126e	0.0	0.0	0.0	0.0	0.0	35.0	0.0
DZW1126f	0.0	0.0	0.0	0.0	0.0	39.4	0.0
DZW1126g	0.0	0.0	0.0	0.0	0.0	63.0	0.0
DZW1126h	0.0	0.0	0.0	0.0	0.0	25.7	0.0
DZW1127a	0.0	0.0	0.0	0.0	0.0	37.8	0.0
DZW1127b	0.0	0.0	0.0	0.0	0.0	50.9	0.0
DZW1004a	0.0	0.0	0.0	0.0	0.0	100.0	0.0
DZW1007ka	0.0	0.0	0.0	0.0	0.0	37.7	0.0
DZW1007b2	0.0	0.0	0.0	0.0	0.0	48.0	0.0
DZW1007f	0.0	0.0	0.0	0.0	0.0	62.3	0.0
DZW1007h	0.0	0.0	0.0	0.0	0.0	36.2	0.0
DZW0402g	0.0	0.0	0.0	0.0	0.0	42.9	0.0
DZW0716b	0.0	0.0	0.0	0.0	0.0	55.3	0.0
DZW0716p	0.0	0.0	0.0	0.0	0.0	27.9	0.0
HSI 68-07	0.0	0.0	0.0	0.0	0.0	10.3	0.0

Bt5

PI-554-104	0.0	8.2	0.0	0.0	0.0	0.0	0.0
Promesse	0.0	17.1	0.0	0.0	0.0	0.0	0.0
Globus	4.8	73.1	0.0	0.0	3.2	16.9	0.0
Starke NIL Bt5	0.0	17.4	0.0	0.0	2.2	0.0	0.0
Tommi	0.0	81.8	0.0	0.0	0.0	12.1	0.0
Blf	0.0	18.0	0.0	0.0	0.0	0.0	0.0
SegQue-1	0.0	27.1	4.8	0.0	0.0	0.0	2.3
SegQue 2	0.0	47.7	0.0	2.4	6.3	0.0	0.0
SegQue 3	0.0	8.6	2.2	5.4	0.0	0.0	0.0
SegQue 4	0.0	38.6	0.0	0.0	0.0	0.0	0.0
SegQue 5	0.0	25.0	0.0	0.0	10.4	0.0	0.0
QueGlo 13	0.0	24.1	2.4	0.0	0.0	0.0	0.0
QueGlo 2	2.0	7.0	0.0	0.0	2.4	3.9	0.0
PerWin	0.0	10.3	0.0	0.0	0.0	0.0	0.0
DZW0510e30	0.0	6.3	0.0	0.0	0.0	0.0	0.0
DZW0405e0	0.0	68.4	0.0	0.0	0.0	0.0	0.0
DZW0605e	0.0	33.3	0.0	0.0	0.0	11.8	0.0
BIFo 5	0.0	13.5	0.0	0.0	0.0	0.0	0.0
CCP 15	0.0	10.0	0.0	0.0	0.0	0.0	0.0
CCP 42	0.0	27.3	0.0	0.0	0.0	0.0	0.0
CCP-27	0.0	37.9	0.0	0.0	0.0	0.0	0.0
GloCor 20	0.0	36.4	0.0	0.0	0.0	0.0	0.0
GloTat 11B	0.0	30.3	0.0	0.0	0.0	0.0	5.1
GloTat 3A	0.0	40.0	0.0	0.0	0.0	0.0	0.0
GloTat 3B	0.0	52.0	0.0	0.0	0.0	0.0	0.0
GloTat 3C	0.0	22.0	0.0	0.0	0.0	2.3	0.0
LahYp10	0.0	22.0	0.0	0.0	0.0	0.0	0.0
PerHyp 17	0.0	40.9	0.0	0.0	0.0	0.0	0.0
QueGlo 13	0.0	26.1	0.0	0.0	0.0	0.0	0.0
QueGlo 9	0.0	25.0	0.0	0.0	7.7	0.0	0.0
SePen 17	0.0	16.7	0.0	0.0	0.0	6.3	0.0
SePen 6B	0.0	45.5	0.0	0.0	0.0	0.0	0.0
SePen 9	0.0	16.0	0.0	0.0	6.3	0.0	0.0
SkaLa 18A	0.0	11.4	0.0	0.0	2.4	0.0	0.0
TomBut 20	0.0	68.4	0.0	0.0	0.0	4.3	3.4
TomHyp 16	0.0	22.5	0.0	0.0	0.0	0.0	0.0
HSI 1010-12	0.0	30.4	0.0	0.0	0.0	2.2	0.0
HSI 676-11	0.0	20.0	0.0	0.0	0.0	2.2	0.0
HSI 186-13	0.0	6.3	0.0	0.0	0.0	0.0	0.0
CP 88.4	0.0	68.2	1.7	0.0	0.0	0.0	0.0
CP 91.6	0.0	83.3	0.0	0.0	0.0	0.0	0.0
CP 88.7	0.0	89.7	0.0	0.0	0.0	2.4	0.0
CP 71.1	0.0	61.2	0.0	0.0	0.0	5.0	0.0
CP 87.5	0.0	30.0	0.0	2.8	0.0	9.1	0.0
CP 86.2	0.0	50.0	0.0	0.0	2.8	2.8	0.0
CP 75.2	0.0	47.1	0.0	0.0	0.0	4.5	0.0
CP 95.4	0.0	77.1	0.0	0.0	0.0	6.3	0.0
CP 78.3	0.0	63.6	0.0	0.0	0.0	0.0	0.0
CP 73.2	0.0	25.0	0.0	0.0	0.0	0.0	0.0
CP 77.2	0.0	61.9	0.0	0.0	0.0	0.0	0.0
CP 81.5	0.0	72.2	0.0	0.0	0.0	3.8	0.0
CP 95.2	0.0	44.8	0.0	3.8	0.0	3.2	0.0
CP 74.1	0.0	81.3	0.0	0.0	0.0	5.4	0.0
Hoherhaemer Bt5	0.0	20.0	0.0	0.0	0.0	0.0	0.0
SePen 20	0.0	7.4	0.0	0.0	4.3	2.4	0.0

Bt13

Thule III	0.0	0.0	2.4	4.3	0.0	0.0	5.2
SegThul 1	0.0	0.0	0.0	0.0	0.0	0.0	7.1
SegThul 2	0.0	0.0	0.0	0.0	0.0	0.0	7.7
SegThul 3	0.0	0.0	0.0	0.0	0.0	0.0	35.7
SegThul 4	0.0	0.0	0.0	0.0	0.0	0.0	12.1
SegThul 5	0.0	0.0	0.0	0.0	2.4	0.0	7.1
SegThul 6	0.0	0.0	0.0	32.0	39.1	28.1	43.5
SegThul 7	0.0	0.0	0.0	2.4	0.0	0.0	16.3
SegThul 8	0.0	0.0	0.0	27.0	23.2	51.4	46.9
SegThul 9	0.0	0.0	0.0	0.0	0.0	0.0	5.0
SegThul 10	0.0	0.0	0.0	0.0	0.0	0.0	23.5
SegThul 11	0.0	0.0	0.0	0.0	0.0	0.0	41.3
SegThul 12	0.0	0.0	0.0	4.3	0.0	0.0	26.9
SegThul 13	0.0	0.0	0.0	0.0	0.0	0.0	35.7
SegThul 14	0.0	0.0	0.0	0.0	0.0	0.0	14.1
SegThul 15	0.0	0.0	0.0	0.0	0.0	0.0	3.4
SegThul 16	0.0	0.0	0.0	0.0	0.0	0.0	7.9
SegThul 17	0.0	0.0	0.0	0.0	0.0	0.0	28.9
SegThul 18	0.0	0.0	0.0	12.1	0.0	2.2	15.6
SegThul 19	0.0	0.0	0.0	0.0	0.0	0.0	24.1
SegThul 20	0.0	0.0	0.0	0.0	0.0	0.0	17.5
SegThul 21	0.0	0.0	0.0	2.4	0.0	0.0	41.7
SegThul 22	0.0	0.0	0.0	0.0	0.0	0.0	9.5
SegThul 23	0.0	0.0	0.0	0.0	0.0	0.0	15.6

Bt2

Skotte	0.0	0.0	69.5	69.5	76.5	0.0	0.0
Inthaler	0.0	0.0	7.0	3.8	14.0	0.0	0.0
Solsice	0.0	0.0	69.8	65.5	70.7	0.0	0.0
Complet	0.0	0.0	26.9	33.3	24.1	0.0	0.0
Hereward	0.0	0.0	37.3	41.5	32.6	0.0	0.0
Ochre	0.0	0.0	25.5	12.0	20.8	0.0	0.0
SegQue Veksel	0.0	2.8	47.1	35.5	20.0	0.0	0.0
ButQue 1	0.0	0.0	39.6	78.0	59.3	0.0	0.0
ButQue 2	0.0	0.0	15.7	10.0	7.0	0.0	0.0
ArPG 1	0.0	0.0	13.0	27.3	11.5	0.0	0.0
ArPG 2	0.0	0.0	3				

Reaction of Czech winter wheat varieties to eyespot

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Abstract

Twelve winter wheat varieties registered in the Czech Republic were tested for their susceptibility to eyespot. Infections were provoked in small field plots by artificial inoculation with *Oculimacula yallundae* and *O. acuformis* in 2017 and 2018. Presence of the *Pch1* resistance gene was checked by the molecular STS marker *Xorw1*. Lowest infection levels were observed for varieties 'Annie' and 'Rebell', both carrying the *Pch1* gene.

Keywords

Oculimacula acuformis · *Oculimacula yallundae* · resistance gene *Pch1* · *Triticum aestivum*

Introduction

The long and warm autumn in the last years and predominance of cereals have caused a high occurrence of eyespot. The disease is caused by *Oculimacula yallundae* (Wallwork & Spooner) Crous & W. Gams and *O. acuformis* (Nirenberg) Y. Marín & Crous. *O. yallundae* and *O. acuformis* differ in pathogenicity, occurrence or sensitivity to fungicides. However, both species may occur on the same stem, they have a similar life cycle and it is not possible to distinguish the symptoms caused by these two species on plants. Even the microscopic differences between *O. yallundae* and *O. acuformis* are not always well discernible. Therefore, species-specific primers (Walsh *et al.* 2005) are useful for the determination of *Oculimacula* spp. incidence by PCR. Based on the PCR analysis, it has been previously shown, that *O. yallundae* prevails over *O. acuformis* in the Czech Republic. Sometimes both *Oculimacula* species occurred together on one stem (Palicová & Matušinsky 2019).

Eyespot can cause yield losses up to 50 %. Eyespot control is based on early treatment at stage BBCH 31-32, but it is not always effective. Recently, resistance of *Oculimacula* spp. strains to fungicides has been increasingly observed. In the monitoring 2015-2017, where 114 *Oculimacula* spp. isolates were evaluated using the agar dilution method for the resistance to prochloraz (Dyer *et al.*

2000), 34 isolates showed low resistance, 31 medium resistance and 1 isolate was highly resistant.

For these reasons, growing of resistant varieties should be taken into account as an effective, economical and environment friendly measure of eyespot control. So far, three major genes of resistance to eyespot have been described. The resistance gene *Pch1* is the most effective one. Previously, detection of *Pch1* in winter wheat varieties using the STS (sequence tagged site) marker *Xorw1* has been performed (Leonard *et al.* 2008), producing a 183bp PCR product in *Pch1* carrying genotypes such as 'Annie', 'Beduin', 'Bonanza', 'Hermann', 'Iridium', 'Manager', 'Pankratz', 'Partner', 'Princes' and 'Rebell' (Dumalaso *et al.* 2015).

Material and methods

The reaction of 12 winter wheat varieties registered in the Czech Republic in trials with eyespot infection was evaluated in 2017 and 2018. The results were compared with the presence or absence of *Pch1* using the *Xorw1* STS marker.

The wheat cultivars were inoculated with *O. yallundae* and *O. acuformis* and tested in a small plot trial in Prague-Ruzyně. The seed originated from the Central Institute for Supervising and Testing in Agriculture, Brno. The inoculum was prepared as a mixture of two isolates of *O. yallundae* and one isolate of *O. acuformis*. The species has been previously determined by PCR. All isolates were obtained from Kroměříž, South Moravia. The fungi were grown on sterilized barley grains. The wheat varieties were sown in deep seedbeds (15×1.5 m) in October 2016 and 2017, with 6 rows per variety. The inoculum was evenly spread within the plants in a dose 40 g/m² two times, in November and in March.

The level of infection was evaluated at growth stage BBCH 73-77 using a 0 to 5 scoring scale (0: no symptoms; 1: one small spot; 2: more spots covering at most a half of the stem perimeter; 3: spots covering more than half of the stem perimeter; 4: spots covering the whole stem perimeter; 5: broken stem). Fifteen randomly selected plants with four stems each were assessed.

Table 1: Reactions of winter wheat cultivars to inoculation with *Oculimacula* spp. in Prague-Ruzyně, 2017 and 2018.

Variety	Level of infection		<i>Pch1</i> (<i>Xorw1</i>)	Year of release	Pedigree
	2017	2018			
Annie	1.13	0.37	yes	2014	Meritto / CH 11.12772 / Eurofit
Arkeos	2.65	0.92	-	2011, FR	-
Bohemia	2.15	1.13	no	2007	540i / U6192 // 540i / Kontrast
Dagmar	2.93	1.20	no	2012	Apache / Nela
Genius	2.68	1.55	no	2014	ACK3094 / 00/412
Julie	2.72	1.93	no	2014	Meritto / Caphorn
Matchball	2.32	1.08	no	2013	Carenius / Boomer
Patras	2.82	1.28	no	2013	Paroli / Toras
Rebell	1.22	0.27	yes	2013, DE	-
Tobak	2.38	1.77	no	2013	Elvis / Drifter // Koch
Turandot	2.70	0.55	no	2012	7125a / S1737-97
Vanessa	2.40	1.20	no	2013	SG-S2040-97 / Rapsodia

Results and discussion

Higher infection levels on the tested varieties were recorded in 2017 (Table 1). Susceptibility to eyespot was observed for the majority of the evaluated varieties. A lower severity of eyespot (and/or stem-base diseases) was observed for varieties 'Annie' and 'Rebell', both possessing the resistance gene *Pch1*.

'Annie' has a lower yield performance, however its bread-making quality is excellent, i.e. class E, and its multiplication area in the Czech Republic was 1.88% in 2018. 'Rebell' occupied a multiplication area of 1.94% in 2018, has a good bread-making quality (class A) and shows also a lower infection by dwarf bunt (*Tilletia controversa*).

Currently, several varieties carrying the *Pch1* resistance gene according to analyses with the molecular marker *Xorw1* are registered in the Czech Republic, e.g. 'Beduin', 'Bonanza', 'Hermann', 'Pankratz', 'Partner', 'Princes'. Although, we didn't test their reaction to eyespot in the small plot field trials, they may also present a promising level of eyespot control. 'Bonanza' has a high yield, its multiplication area was 1.26% in 2018, however, its baking quality is inferior (i.e. class C). 'Pankratz' has a high yield and good baking quality (i.e. class A). Its multiplication area in 2018, however, was only 0.27%. For 'Partner', a class B wheat, the multiplication area in 2018 was only 0.11%, although it has also high yields.

Evidently, the presence of the *Pch1* gene doesn't necessarily mean a penalty for grain yield and bread-making quality. Also the multiplication areas of some varieties with lower levels of eyespot infection in the Czech Republic in 2018 were not inconsiderable.

Acknowledgements

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QTL analysis of resistance to the fungal pathogens *Blumeria graminis*, *Zymoseptoria tritici*, and *Pyrenophora tritici-repentis* using a winter wheat multiparent advanced generation intercross population

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Bread wheat (*Triticum aestivum* L.) is one of the most important food crop species in the European Union. Breeding of new varieties resistant to fungal pathogens is an ongoing task, especially in today's era of sustainable agriculture. Simultaneous genetic analysis of resistance to multiple diseases with high accuracy can be achieved using multiparent advanced generation intercross (MAGIC) populations. Such populations provide improved accuracy of quantitative trait loci (QTL) mapping via the genetic recombination captured over several rounds of intercrossing. Furthermore, a MAGIC design facilitates analysis of numerous disease traits within a single population due to the potentially high numbers of alleles contributed by multiple founders. In the present study, we report the construction of a genetic linkage map for our eight-founder Bavarian MAGIC winter wheat population (BMWpop) and the genetic mapping of resistance loci for the three fungal diseases powdery mildew (PM), Septoria tritici-blotch (STB), and tan spot (TS).

The BMWpop comprising 394 F_{6,8} recombinant inbred lines (RILs) was developed using the eight founders 'Event', 'BAYP4535', 'Ambition', 'Firl3565', 'Format', 'Potenzial', 'Bussard', and 'Julius'. The lines were intercrossed over several rounds of crossing using a greatly reduced MAGIC mating design with an additional eight-way intercross step. The BMWpop was genotyped with a 15K + 5K Infinium® iSelect® single nucleotide polymorphism array and a functional marker for the powdery mildew resistance gene *Pm3a*. Population structure was investigated using principle coordinates based on Roger's distance matrix in R/ape V5.0. The genetic linkage map was constructed using the R packages 'mpMap' V2.0.2 and 'mpMap2'. The genetic distances were calculated according to Haldane. The founder contribution was determined using a threshold of 0.7. Resistance to PM and STB was assessed in field trials located in Germany over two years. TS disease severity was scored in Germany in two years and in Denmark in 2017. PM infection took place naturally, whereas STB and TS trials were inoculated. Adjusted means fitting the genotype as fixed effect were used as phenotypic input data for QTL detection. QTL analysis was carried out using R/mpMap without the consideration of cofactors. A

genome-wide QTL significance threshold of $\alpha < 0.001$ was derived from an empirically null distribution over 1000 simulation runs.

The first two principle coordinates explained a low proportion of the molecular variation (2.7% and 2.3%, respectively), indicating mild population structure in the BMWpop. All eight founders contributed genetic material to each chromosome, except for chromosome 4D. The founder probabilities were equally distributed for almost all chromosomes. In total, 5436 markers were mapped to all 21 wheat chromosomes representing 2804 unique sites across the genome. The total chromosome length spanned a distance of 5230 cM with individual chromosome length ranging from 87.0 cM (4D) to 389.6 cM (7D). Based on the above mentioned threshold, a mean recombination event per RIL of 73 was determined. The population parameters analyzed indicated that the BMWpop and the associated genetic linkage map represents a valuable genetic resource for wheat genetic analysis. The residuals of the adjusted means of all three disease traits followed a normal distribution. The heritability estimate for PM, STB, and TS was 0.6, 0.8, and 0.7, respectively. STB was phenotypically positively correlated with PM and TS, however, the latter two did not correlate. QTL analysis of all disease traits identified 22 genetic loci. For PM, six QTL were mapped to chromosomes 1A, 3D, 5A, 6B, 7A, and 7D with a total explained phenotypic variance (R^2) of 0.3. The QTL on chromosome 1A coincided with a functional marker for the *Pm3a* gene. Genetic analysis of STB identified seven QTL on chromosomes 1A, 1B, 2B, 2D, and 4B, with a total R^2 value of 0.3. Nine QTL controlling resistance to TS were detected on chromosomes 1A, 2A, 2B, 2D, 3D, 4B, 5B, and 7A explaining 0.4 of the total phenotypic variation. On chromosomes 1A and 2B, the support interval of QTL for STB and TS overlapped (Figure 1), suggesting that the same genetic locus may be associated with reaction to both necrotrophic pathogens. On the short arm of chromosome 1A, the PM QTL was located 20 cM distally to the STB and TS QTL (Figure 1), however, the additive effects of the founders indicated that breeding for simultaneous resistance to all three diseases appears probable when considering this genetic region.

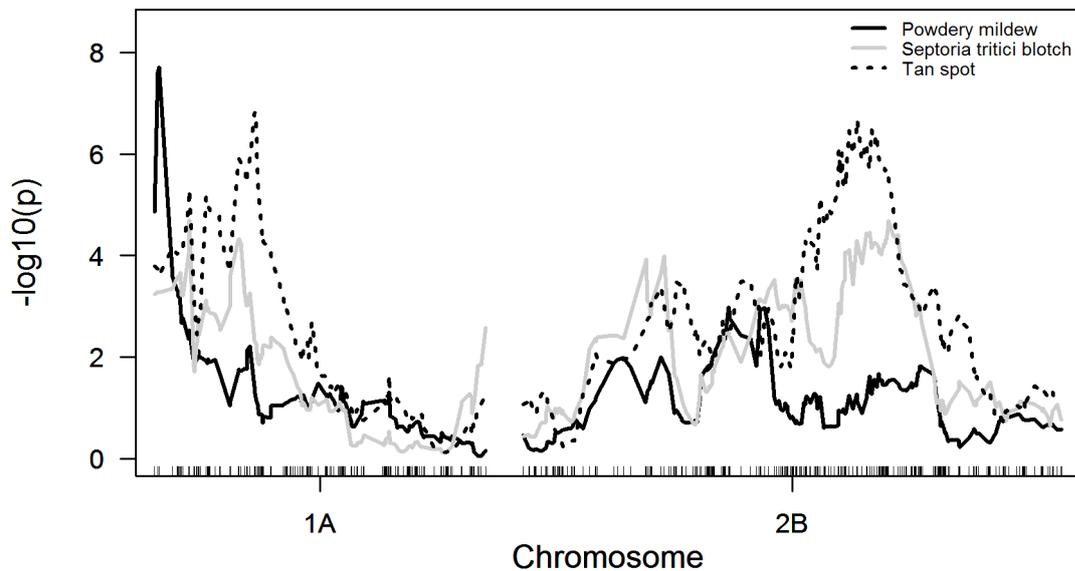


Figure 1: QTL on chromosomes 1A and 2B for disease resistance to powdery mildew (black, solid line), Septoria tritici blotch (grey, solid line) and tan spot (black, dotted line).

Keywords

BMWpop · MAGIC population · powdery mildew · QTL · Septoria tritici blotch · tan spot · *Triticum aestivum*

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Association analysis for identifying relationships between molecular markers and severity of diseases

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Abstract

Association analysis can be used to identify relations between traits of interest and genetic markers. In this study, 276 wheat (*Triticum aestivum* L.) genotypes were screened for diseases such as leaf rust, yellow rust, Fusarium head blight and powdery mildew in the field and were genotyped with 71 iPBS markers. Analysis of genetic variation revealed 194 polymorphic locations. Wheat genotypes were assigned to five groups based on population genetic structure. Association analysis with MLM and GLM methods identified that six, five, five and five markers were associated with QTL for leaf rust, yellow rust, Fusarium head blight and powdery mildew, respectively.

Introduction

Wheat, a cereal of the Gramineae (Poaceae) family and of the genus *Triticum*, is the world's largest cereal crop. It is an important staple food of many countries and occupies a unique position used for the preparation of a wide range of food products. Identification of genotypes with desirable traits and their subsequent use in breeding programs and the establishment of suitable selection criteria are necessary for the improvement of varieties. Association analyses are based on the linkage disequilibrium (LD) between loci (Pritchard *et al.* 2000). In case of a tight linkage between the marker and the resistance locus, it is possible to localize the resistance gene by genotyping neighboring markers. The difference in allele or genotype frequencies of a marker can be observed between the case and control samples if the marker locus itself is causing the disease or is in linkage with the susceptibility/resistance locus (Risch & Merikangas 1996).

The present study was undertaken to identify the genomic region associated with four diseases, *i.e.* leaf rust, yellow rust, Fusarium head blight and powdery mildew in wheat with the following specific objectives: (i) phenotyping the variability of leaf rust, yellow

rust, Fusarium head blight and powdery mildew resistance/susceptibility in diverse wheat germplasm; (ii) genotyping the wheat germplasm with iPBS markers (Kalendar *et al.* 2010) to determine the population structure; and (iii) the identification of marker-trait association through LD based association mapping.

Material and methods

A total of 276 genotypes were used for the investigation of genetic diversity. Approximately 5 g of fresh leaf material was pooled from seedlings of each genotype for DNA extraction.

iPBS bands were scored for presence (1) or absence (0) of polymorphic bands. Only clearly distinguishable bands were checked and transformed into 0/1 binary character matrix. The dendrogram was constructed using simple matching (SM) coefficient. Complete linkage clustering was chosen because it maintained a fairly consistent topology over the various distance matrices that were examined. In addition, a cophenetic value matrix was calculated and compared with the original similarity matrix to test the goodness of fit of the cluster analysis and compare the clustering method. The polymorphic information content (PIC) value for each primer combination was calculated. Analysis of population structure among the 276 genotypes was performed using software package STRUCTURE vers. 2.2 (Evanno *et al.* 2005). The optimum number of clusters (populations) or K number was selected after eight independent runs of a burn-in of 100 000 iterations and 100 000 MCMC repetitions for each value of K (testing from K=2 to K=10), using no prior information and assuming correlated allele frequencies and admixture. The optimum number of clusters (K) was estimated by computing the *ad hoc* statistic DK based on the rate of change in the log probability of data between successive K values.

The mean phenotypic values were used for association analysis. Four diseases, *i.e.* leaf rust, yellow rust, Fusarium head blight and

powdery mildew were analyzed. Four methods were used to test the associations between iPBS markers and evaluated traits using the TASSEL software vers. 4.1.20 (Bradbury *et al.* 2007).

Results and discussion

Within the investigated population of 276 wheat genotypes, a total of 446 bands were produced and 194 bands were shown to be polymorphic in the range of 120 to 630 bp. The number of polymorphic fragments per primer ranged from 3 (iPBS2278) to 10 (iPBS2393, iPBS2226, iPBS2246 and iPBS2255), with an average of 2.73. The highest PIC was observed in iPBS2374. The average value for PIC was 48. STRUCTURE analysis revealed five clusters (K) that maximized the DK parameter. The 53, 50, 53, 52 and 16 genotypes were assigned to group I, II, III, IV and V. Also, 52 admixed genotypes were made up.

Based on results of the forth model that used both factors of Q and K, there are eight markers that can be considered to be the most interesting candidates for further study. These are iPBS2217 C and iPBS2225 A for yellow rust, iPBS2074 B for Fusarium head blight, iPBS2383 D, iPBS2276 B and iPBS2218 B for leaf rust, and iPBS2255 A and iPBS2271 A for powdery mildew. These markers would provide a useful target for future breeding programs such as MAS.

Although marker validation by testing for the presence of the marker on a range of cultivars and other important genotypes is a necessary requirement, the identified markers that showed strongest effects on the traits provide ideal candidates for further study or future inclusion in marker assisted selection (MAS). Based on results of the forth model that used both factors of Q and K, there are nine markers that can be considered to be the most interesting candidates.

In this study, iPBS marker were used for evaluation of genetic variation and association analysis and it is worth mentioning that although iPBS markers are very useful for linkage mapping, these markers need to be converted to sequence characterized amplifi-

ed region (SCAR) markers. Those are an ideal choice for MAS, because they are detected by single genetically defined loci, are identified as distinct bands in agarose gels, are easier to score, are less sensitive to reaction conditions, and are more reproducible. After the identification of linked markers and marker validation, it is to be determined whether linkage mapping has a resolution high enough. Then, markers can be reliably used for MAS. Obviously depending on the environment, QTL must be tested for identification of the most stable QTL (Bernier *et al.* 2007) that is needed to study more experiments across multi environments.

Keywords

iPBS marker · QTL · *Triticum aestivum* · wheat

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Molecular breeding for stem rust resistance in winter rye

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Resistance to stem rust in rye (*Puccinia graminis* f. sp. *secalis*) is a largely unstudied topic compared to its close relative stem rust in wheat (*P. graminis* f. sp. *tritici*), even though it can cause grain yield losses of up to 60% in epidemic years. In a two-year CORNET project we studied the genetics of the resistance by classical QTL mapping, phenotypic selection and a case-control approach.

For QTL mapping, four biparental populations composed of inbred lines ($F_{2:3}$, $F_{2:4}$, $F_{2:5}$, BC_{1S_2}) were phenotyped with 68-70 progenies per population in artificially inoculated field trials in single rows in three locations and two years. They were genotyped with a 10k SNP array. We could identify both quantitative and qualitative inheritance (Fig. 1). For the latter, a locus at the distal end of chromosome 7R was found in two independently developed populations and explained 73 and 91 percent of the phenotypic variation (adj. R^2), respectively. From the 3:1 (resistant:susceptible) segregation ratio of the heterozygous genotypes within the single rows we concluded a dominant gene action. The explained variance of the quantitative inheritance was much smaller resulting in an adjusted R^2 of 0.47 in a linear model with 2 QTL. In an additional test in the seedling stage (leaf-segment test) we could show that the quantitative resistance is active in the adult plant stage only, compared to the single resistance gene on chromosome 7R that is effective in all plant stages.

In previous experiments, full-sib families (FSF) were developed by intercrossing two individual resistant plants from different genetic resources (self-incompatible genotypes) in a pairwise pattern. The “improved” FSF were tested in the recent project. Per plot, 15 single plants of each FSF were scored individually (in all locations). We observed that the FSF performed better than two adapted German population varieties consisting almost entirely of susceptible plants. No FSF, however, had fully resistant material only.

Making use of this segregation in the genetic resources, we tried to directly map genes/QTL in five self-incompatible populations. Genotypes were clustered into resistant and susceptible by a leaf-segment test and a 10k SNP array was used for genotyping. In a so-called case-control study (contingency-table-based test statistics), significant markers could be identified in some populations. Marker based population structure made population-wise analysis necessary. This was a drawback, because individual populations were rather small (73 genotypes per population) and unequal ratios of resistant and susceptible plants showed spurious significance of markers. Nevertheless, some markers of all tested populations will be validated in field test.

This project resulted in new resistant material, gene-linked markers and new methods to find genes in genetic resources directly.

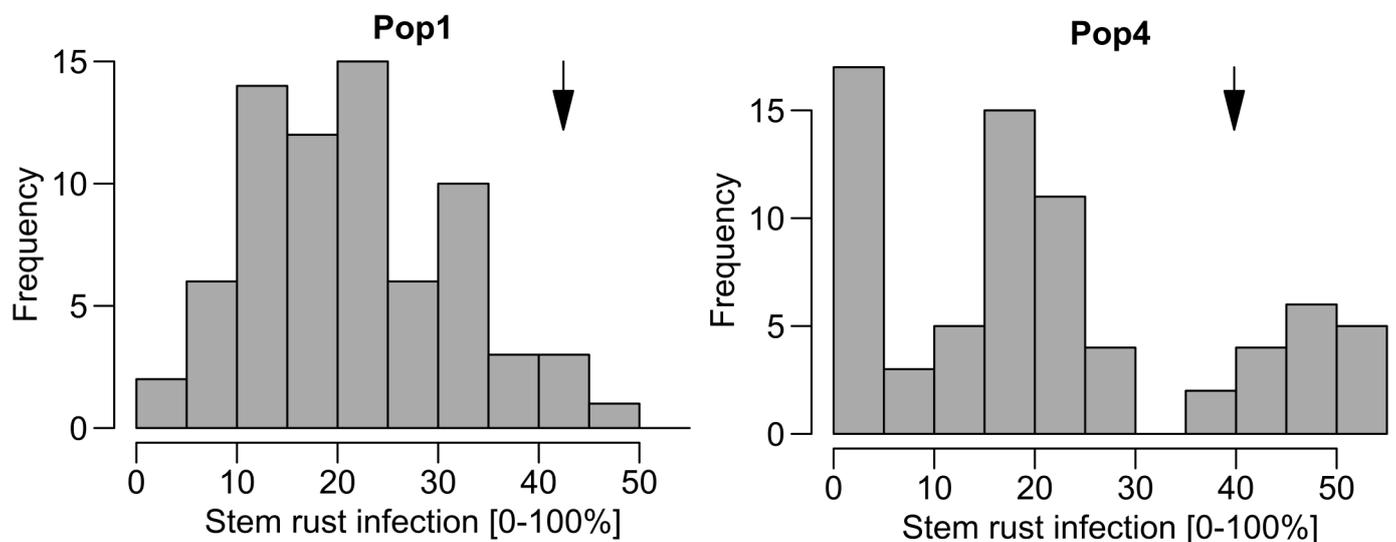


Figure 1: Phenotypic distributions of two biparental populations with quantitative (Pop1) and qualitative (Pop4) inheritance. Stem rust infection level of the susceptible parent is indicated by an arrow.

Keywords

Case control · genetic resources · leaf segment test · *Puccinia graminis* · QTL mapping · *Secale cereale*

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Influence of isolate, host genotype, and environment on the ergot reaction of winter rye

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Ergot, a fungal disease of cereals, is caused by *Claviceps purpurea* and can infect more than 400 grass species with a preference of outcrossing grasses such as rye (*Secale cereale* L.). After an infection, alkaloid containing and poisonous black-purpled sclerotia are formed on the ear of the host plant. Ergotism, initiated by ergot alkaloids and resulting in gangrene, neurological diseases and finally death, occurred frequently in the early Middle Ages as an epidemic disease. Nowadays, contamination with ergot is reduced drastically due to cleaning procedures and implementation of regulation measures. The limits are set to 0.05 wt% for human consumption and 0.1 wt% for animal feeding. However, in years with unfavorable weather conditions, such as cold and rainy weather during the infection process, the contamination with ergot can be higher. Therefore, ergot is still a problem these days, especially in open pollinating hybrid cereals such as winter rye. For that reason, we analyzed within the scope the CORNET project 'NoErgot' the influence of isolate, cultivar and environment on the ergot reaction of winter rye.

The study consists of two experimental set-ups. In experiment 1, ergot severity (%) and anther rating (1-9) was recorded from 16 winter rye cultivars at 10 locations in three countries, *i.e.* Braunschweig, Oberer Lindenhof, Wohlde, Petkus, Wulfsode, Kleptow (Germany), Hagenberg, Zwettl (Austria), Koscielna Wies and Zybiszow (Poland), after artificial inoculation by *C. purpurea* with three isolates of different origin (AT, DE, PL). For experiment 2, ergot severity (%) and anther rating (1-9) of 25 factorial crosses of 5 seed parents and 5 pollen parents were gathered after artificial inoculation by *C. purpurea* at 5 locations, *i.e.* Oberer Lindenhof, Wohlde, Petkus (Germany), Koscielna Wies and Zybiszow (Poland).

The first experiment evaluated the diversity due to genotype, isolate and environment (Fig. 1). The percentage of ergot in grain ranged on average from 0.07% to 9.69% across locations, indicating a high variation in disease severity. Heritabilities of all experiments was moderate (0.62) to high (0.93-0.97), indicating a well suited experimental set up. Two locations, *i.e.* Hagenberg and Oberer Lindenhof, showed a very high infection level compared to

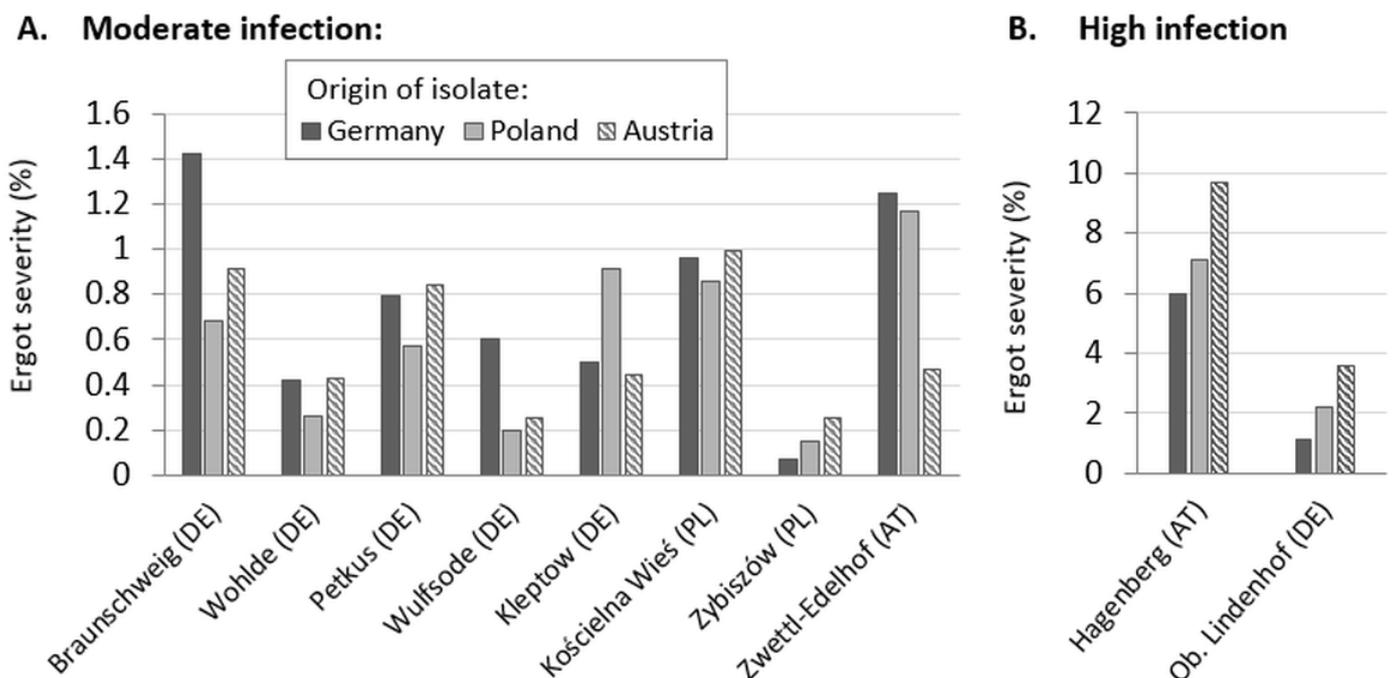


Figure 1: Mean of ergot severity (%) of 16 winter rye cultivars after artificial inoculation by *Claviceps purpurea* for three isolates of different origin and in three different countries (AT, Austria; DE, Germany; PL, Poland)

the remaining eight locations. The high variation between genotypes and environments revealed the importance of testing genotypes across environments. No interaction occurred between genotypes and isolates as it is typical for quantitative pathosystems. Obviously, the aggressiveness of the isolate must not be considered with a high effort in future testing systems.

The second experiment evaluated the relative importance of ergot susceptibility of female parents and pollen-fertility restoration ability of male parents and their interaction on the ergot reaction of rye hybrids. The male component, interaction of male with environment and male×female interaction, *i.e.* specific combining ability were significant ($P>0.01$). Interaction of female with environment was of lower importance ($P>0.05$). Significant ($P>0.01$) differences among the pollen-fertility of individual pollinators occurred. A significant negative correlation of $r \geq -0.63$ between ergot severity and anther rating was observed in all experiments. Further improvements of ergot resistance by selecting restoration ability is still possible.

Keywords

Alkaloids · *Claviceps purpurea* · *Secale cereale*

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Investigation of net blotch resistance of barley and preliminary data on Hungarian pathotypes of *Pyrenophora teres f. teres*

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In Hungary, barley is the third most important crop with a growing area of ca. 250 000 ha, and net blotch has become one of the major diseases of both spring and winter barleys. It was shown that Hungarian isolates of *Pyrenophora teres f. teres* (PTT), the causal agent of the net form of the net blotch disease, exhibited a high level of genotypic diversity, but there is no available information about the pathotypes present in the local populations of this fungus.

The efficiency of resistance genes depends on the developmental stage of barley and the fungal pathotypes present in the area. Few net blotch resistance sources have been known and the majority of the resistance sources were identified in spring genotypes, although winter cultivars also have a great economic impact.

Plant hormones and antioxidant enzymes have a key role in the activation of plant defence mechanisms. Ascorbate peroxidase (APX) is the key enzymes in the ascorbate-glutathione cycle. Guaiacol peroxidase (GPX) has an outstanding role in the defence mechanisms against pathogens. The activation of the jasmonic acid (JA) signalling pathway is required for resistance against necrotrophic pathogens. The positive or negative interaction between salicylic acid (SA) and JA pathways are known. Little is known about the oxidative burst and hormonal control of defence reaction during infection by PTT. This research is to uncover (i) the net form of net blotch pathogen's local pathotypes and the effective resistance sources against them, (ii) the role of antioxidant enzymes and plant hormones in this pathosystem.

Pathotypes of thirteen monoconidial PTT isolates from symptomatic barley leaves collected at three locations in Hungary were tested using Afanasenko's differentials and five additional varieties. Seedlings were grown in pots and sprayed in two-leaf stage with conidium suspension. Infection response (IR) was scored on the 2nd leaf based on the scale of Tekauz (1985) (avirulent: IR < 5; virulent: IR ≥ 5) on the 10th day after inoculation.

Net blotch resistance of 260 barley varieties (182 winter type, 78 spring) were tested. Field tests were carried out in 2017 and 2018 in Hungary. Plots were inoculated with naturally infected barley straw. The seedling test was carried out as described in the virulence test. Scoring was carried out four times based on the Tekauz scale. Data were used for calculating AUDPC curves.

Salicylic acid/ jasmonic acid was extracted and antioxidant enzyme assays were carried out. Seedlings were sampled on the 1st, 2nd and 3rd day after inoculation.

In total, 99 (38%) of isolate × barley differential combinations were virulent. Each tested isolate was virulent on the two Hungarian commercial varieties 'Botond' and 'BC5', indicating that these cultivars could not be useful to differentiate local PTT pathotypes but might be suitable as susceptible controls. Foreign barley lines showed differential responses from 1 ('Sylphid') to 9 ('Harrington'), and in average four virulent isolates per differential. However, 'Sylphid', CI 5791, CI 4207, CI 9825, CI 11458 and 'Sebastian' showed little differential response. Individual isolates were virulent to 2 to 20 barley differentials (average of 8), the most virulent isolates were H-896 and H-922, accounting for 37% of the virulence found in this study, whereas isolates H-286 and H-779 were only virulent to the two Hungarian cultivars. Further investigations are going on to survey the virulence of Hungarian PTT populations and improve the barley differential set by testing additional cultivars.

The disease severity of adult plants was 3.5 times higher in 2018 than in 2017 in the field, with average AUDPC values of 394 and 82, and a range of 64-623 and 13.72-158, respectively. Although the majority of genotypes proved to be moderately sensitive in both years, the progression of disease was faster in 2018. Ten varieties were resistant (R) or moderately resistant (MR) in both years.

The seedling resistance of barley genotypes was tested against three isolates (H-502/1, H-618, H-774) in the greenhouse. Based on the average reaction scores isolate H-618 was the most virulent, followed by H-502/1 and H-774 with average AUDPC values of 88, 65 and 54, respectively. In case of H-502/1, the normal distribution could be observed while in case of H-618 and H-774 two peaks appeared, which refer to the presence of an effective resistance gene. Five winter barley lines were identified as MR both in the field and in the greenhouse experiments (Fig. 1).

Studying the role of phytohormones and antioxidant enzymes during pathogenesis, significant changes were detected in case of SA, JA, APX and GPX ($p=0.01$), although their tendencies were not clear. Some effect could be observed between the hormones or

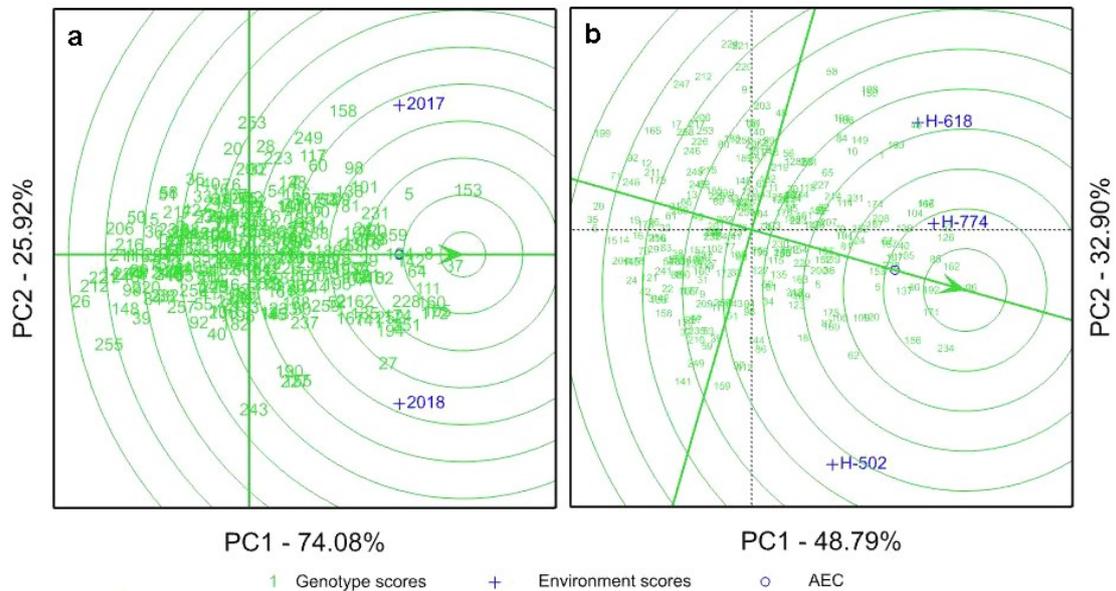


Figure 1: Adult plant and seedling resistance against *Pyrenophora teres* f. *teres* based on AUDPC score of barley genotypes. Five winter barley lines (BC6, BC69, BC116, BC165, BC211) were identified as MR both in the field and greenhouse experiments. GGE biplot analyses revealed significant genotype by year (a) and genotype by isolate (b) interactions and allowed to identify genotypes with stable and/or specific resistance performance.

antioxidant enzymes and the disease severity of genotypes, but there were not significant correlations or clear tendencies among them. Therefore, further examinations of SA/JA and antioxidant enzymes are necessary.

Keywords

Adult plant resistance · antioxidant enzymes · *Hordeum vulgare* · plant hormones · seedling resistance · virulence test

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Genetic analysis of new sources of seedling resistance to powdery mildew and crown rust in oat

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Abstract

The exploitation of genetic resources for the enhancement of cultivated germplasm constitutes a supporting pillar of plant breeding. This is particularly true for resistance breeding due to coevolution of host and pathogen which lowers the diversity of resistance genes in cultivars. Two *Avena byzantina* accessions AVE2406 and AVE2925 were characterized for their resistance to powdery mildew and crown rust. Genetic analysis and quantitative trait locus (QTL) mapping showed that powdery mildew resistance was controlled by a single gene in each accession. A major crown rust QTL was identified in AVE2406. The three resistance loci were placed on the recently established oat consensus map by means of highly similar genotyping-by-sequencing (GBS) markers available among the genetic maps.

Keywords

Avena byzantina · *Blumeria graminis* · disease resistance gene · GBS · genetic mapping · oat consensus map · *Puccinia coronata*

Introduction

Cultivated oat (*Avena sativa* L.) is an important crop for human and animal nutrition. The allohexaploid oat genome ($2n = 6x = 42$, AACDD) is large (12.5 Mbp; Yan *et al.* 2016) and highly rearranged: The A and D genomes mix up in the hexaploid condition because they are very similar and reciprocal chromosomal translocations are frequently observed. Significant genomic resources for oat were established only recently when GBS became available (Huang *et al.* 2014). GBS is an all-in-one approach combining SNP discovery and SNP scoring. This technology allowed establishing the first true road map of the oat genome (Chaffin *et al.* 2016). It is a consensus map developed from twelve biparental populations whose parents carry the most common chromosome configurations.

Powdery mildew and crown rust caused by *Blumeria graminis* f. sp. *avenae* and *Puccinia coronata* f. sp. *avenae*, respectively, are two major fungal diseases of oats. The former is relevant to Northwestern Europe, whereas the latter occurs in all oat-growing

regions. Ten major genes for resistance to powdery mildew have been catalogued (Hsam *et al.* 2014, Herrmann & Mohler 2018), whereas more than 100 resistance genes have been described for crown rust (Nazareno *et al.* 2018). However, many uncharacterized sources for resistance to powdery mildew and crown rust do exist (Herrmann & Roderick 1996, Okoń *et al.* 2014, 2016, 2018, Sowa *et al.* 2016).

Wild oats are useful genetic resources for the enhancement of cultivated oat germplasm. The report reviews recent work on the characterization of two powdery mildew resistance genes derived from *A. byzantina* accessions AVE2406 and AVE2925 (Herrmann & Mohler 2018). In addition, the location on the oat consensus map of a crown rust resistance gene available in AVE2406 is presented.

Material and methods

Plant material

Two *A. byzantina* accessions, AVE2406 from Libya and AVE2945 from Italy, were crossed with two susceptible oat cultivars each (Herrmann & Mohler 2018). F_{2:3} lines of the crosses with 'Dominik' were used for segregation analysis of powdery mildew reaction using detached leaf tests in the lab, whereas F_{4:7} and F_{4:8} lines of the crosses with 'Leo' were used for scoring powdery mildew reaction in field trials. AVE 2406 also showed a resistance reaction to crown rust. For this, F_{4:5} lines of the cross with 'Leo' were used for scoring seedling reactions. GBS was carried out for F_{4:5} lines.

Phenotyping

Powdery mildew scoring was described in Herrmann & Mohler (2018). Crown rust seedling reaction tests were carried out in the glasshouse under controlled conditions with a mean relative humidity of 67%, a temperature of 18°C and 16 h of light per day. Inoculation was done on 10-day-old plants using a mixture of crown rust isolates collected in Germany. Disease assessments based on 10 plants per line were made 10 days after inoculation, and followed the 0–4 infection type (IT) scoring system, in which IT '0' indicated no visible symptoms, IT '+' indicated hypersensitive spots, IT '1' indicated small uredinia with necrosis, IT '2' indicated small to medium sized uredinia with green islands and surrounded by ne-

Table 1: Segregation of F_{2:3} lines for powdery mildew reaction (NSR, non-segregating resistant; Seg, segregating; NSS, non-segregating susceptible)

Cross	NSR	Seg	NSS	$\chi^2_{1:2:1}$	P
Dominik/AVE2406	44	112	42	3.45	0.18
Dominik/AVE2925	36	91	44	1.46	0.48

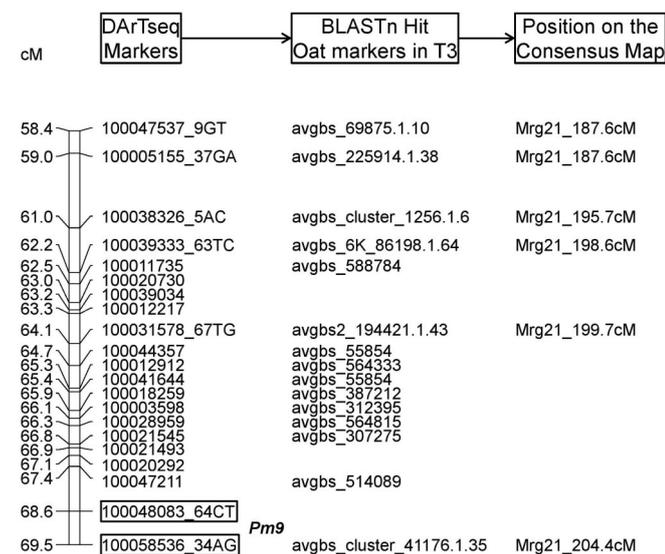


Figure 1: *Pm9* in AVE2406 is located on Mrg 21. Framed markers delimit the QTL peak interval. Absolute map distances are in centimorgan (cM)

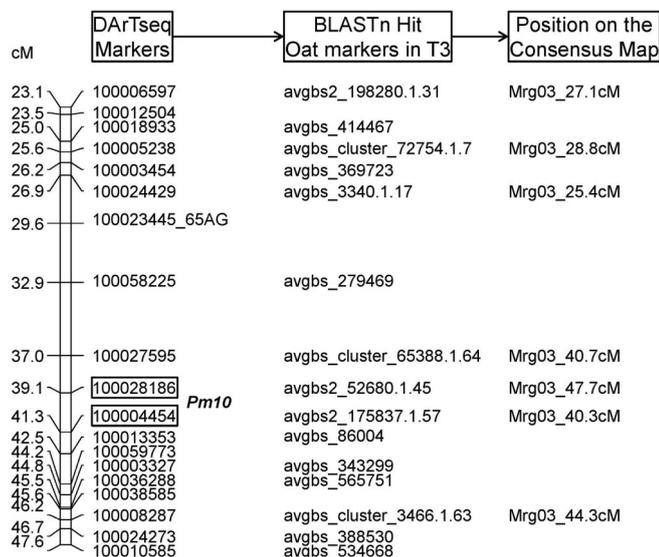


Figure 2: *Pm10* in AVE2925 is located on Mrg 03. Framed markers delimit the QTL peak interval. Absolute map distances are in centimorgan (cM)

crisis or chlorosis, IT '3' indicated medium to large sized uredinia with chlorosis, IT '4' indicated large uredinia without chlorosis. The letter 'C' was used to indicate more than normal chlorosis. Infection types '3' or higher were regarded as compatible, whereas ITs

of '2' or lower were regarded as incompatible. Infection types were converted into a numerical scale.

Genotyping and data analysis

We used DarTseq for genotyping, a GBS service provided by Diversity Arrays Technology P/L company (Bruce, Australia). This platform scores single nucleotide polymorphisms (SNPs) and presence/absence variants (PAVs). Marker pruning and genetic map construction were described in Herrmann & Mohler (2018). Linkage groups were allocated to the oat consensus map by using DarTseq marker sequences as queries against the database Oat markers in T3 (BLASTn; <https://triticeaetoolbox.org/oat/viroblast/viroblast.php>). QTL analysis of resistance to crown rust was carried out as described in Mohler *et al.* (2016), whereas composite interval mapping and point analysis were used for QTL analysis of resistance to powdery mildew (Herrmann & Mohler 2018).

Results and discussion

The genetic map of Leo/AVE2406 comprised 2516 markers distributed over 30 linkage groups, while for Leo/AVE2925 921 markers were allocated to 30 linkage groups (Herrmann & Mohler 2018). Since both maps shared 318 markers, a total of 3119 markers were used as queries against the database Oat markers in T3. A number of 72% of the DarTseq markers showed high similarity (bit score >100) to the GBS markers of the consensus map and only 0.3% showed no hit at all. In relation to the absolute numbers of SNPs and PAVs mapped in Leo/AVE2406 and Leo/AVE2925, the proportion of the SNP-containing sequences that showed similarity to the GBS markers available in the database was greater (96% SNPs compared to 63% PAVs). All linkage groups but one that consisted of three marker loci could be assigned to the oat consensus map.

The powdery mildew reaction tests in the lab showed that the number of non-segregating resistant, segregating and non-segregating susceptible F_{2:3} lines in both populations approximated a 1:2:1 ratio indicating single-gene segregation (Table 1). Powdery mildew reaction data from field trials did not show a bimodal distribution in advanced lines of both populations although a single gene each could be assumed. There was an excess of resistant plants – higher for Leo/AVE2925 – possibly because of low or uneven levels of natural inoculum source which caused errors in disease score classification. However, despite the challenges associated with the field trials, the QTL scan across the genome revealed in each population a single significant LOD peak confirming results of detached leaf test. The resistance gene in accession AVE2406, named *Pm9*, was mapped to a linkage group representing merged group (Mrg) 21 of the oat consensus map (Fig. 1). The QTL explained 41% of the phenotypic variance. The gene in accession AVE2925, designated *Pm10*, was located on Mrg 03 (Fig. 2). The phenotypic variance explained by the QTL (16%) was smaller than for *Pm9* showing the impact of level of infection on the phenotypic variance that is attributed to a resistance QTL.

The infection types for crown rust observed in F_{4:5} lines of Leo/AVE2406 were 'C', '2', '3', and '4'. However, the distribution of infection types in the population showed that there were more susceptible than resistant lines (Fig. 3). Here, the small population size and/or sampling bias during population development might be causative for this deviation.

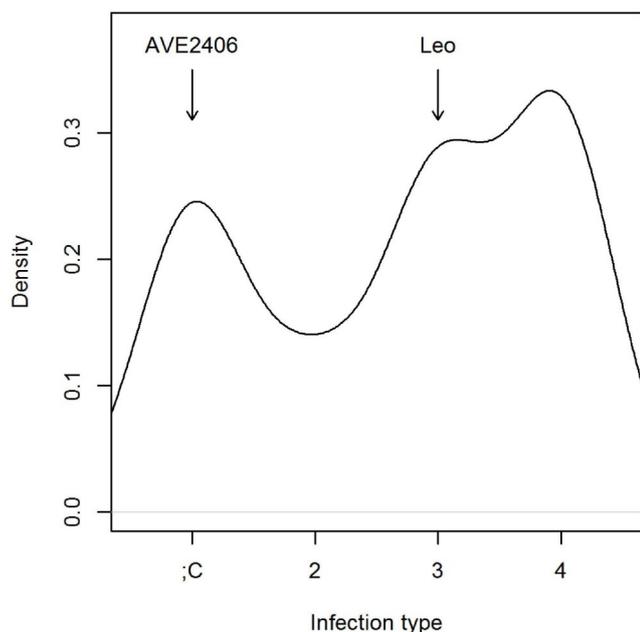


Figure 3: Distribution of crown rust infection types for Leo/ AVE2406 F_{4:5} lines

QTL analysis mapped the crown rust resistance gene to a linkage group corresponding to Mrg 01 of the oat consensus map. The QTL explained 22% of the phenotypic variance. Thus, it appears that there are other undetected QTL for crown rust resistance.

In the present study, we explored the *A. byzantina* accessions AVE2406 and AVE2925 for resistance to powdery mildew and crown rust and located underlying QTL on the oat consensus map by comparing DArTseq and consensus map GBS marker information using BLASTn. The information derived from different GBS platforms is interchangeable provided that DNA sequences along with their map location are lodged in a database such as T3/oat - The Triticeae Toolbox. This prerequisite will allow drawing a clear picture on the location of disease resistance genes in the oat genome. Efforts are underway to map further powdery mildew resistance genes on the oat consensus map which will support the identity of these genes.

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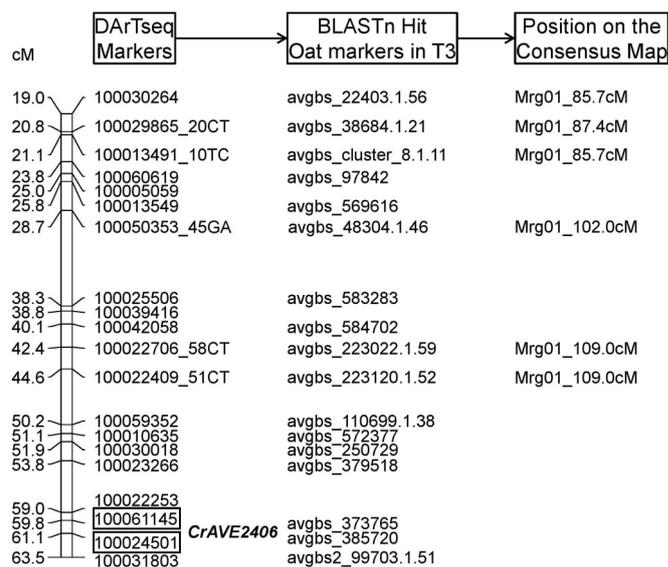


Figure 4: The crown rust resistance gene in AVE2406 maps to Mrg 01. Framed markers delimit the QTL peak interval. Absolute map distances are in centimorgan (cM)

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A transcriptome-based approach for developing breeding lines in *Lolium* sp. with multiple pathogen resistance

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Ryegrass (*Lolium* sp.) is the most important cool-season forage crop in temperate regions. Though, the crop yield and quality is considerably affected by several fungal and bacterial obligate biotrophic pathogens. The overall purpose of this study is directed to developing ryegrass cultivars with multiple pathogen resistance and agronomic adaption to Germany's agricultural conditions. This aim was achieved by combining genes for resistances to stem rust, crown rust and bacterial wilt. Therefore, several segregating mapping populations were created and phenotyped regarding to resistance response. The combination and pyramidisation of resistance genes was accomplished by the use of specific molecular markers, which will be derived by bulked segregant analysis combined with next generation sequencing based massive analysis of cDNA ends (MACE) transcriptome profiling. For this purpose RNA was isolated from bulks of infected and non-infected leaf segments from susceptible and resistant genotypes of various full-sibbling mapping populations ($n \geq 200$) and their respective parental lines for every investigated pathogen. After MACE was performed, bioinformatic analysis detects SNPs and transcripts that were exclusively expressed in the resistant bulk. Thus, 34 molecular markers were genetically mapped to a 50.8 cM spanning region surrounding the stem rust resistance locus *LpPg1*. Three closely

linked ERT markers can be used as a highly reliable and practicable selection tool for stem rust resistance. Furthermore, four potential markers for another stem rust resistance gene *LpPg2* were identified and six markers linked to crown rust resistance *LpPc1* were developed. The developed markers were recently integrated in existing breeding programmes and allow marker-assisted selection of multiple resistances and accelerate the development of ryegrass varieties with high level of pathogen resistance.

Keywords

Bacterial wilt · *Lolium multiflorum* · *Lolium perenne* · MACE · perennial ryegrass · *Puccinia coronata* · *Puccinia graminis* · ryegrass · stem rust · *Xanthomonas translucens*

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Stolbur in potatoes and vegetables

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In the last years a disease has been observed in potatoes and vegetables in eastern Austria, which has not or rarely appeared in the past. Molecular analyses identified stolbur phytoplasma (*'Candidatus Phytoplasma solani'*) as cause of the disease. This pathogen has been observed in Austrian vineyards for decades, where it induces *'Bois Noir'*. As phytoplasma, *'Ca. Phytoplasma solani'* is a cell wall-less, small (<1 µm) bacterium (Mollicutes) with a small genome (<1000 kb). The bacterium has a dual host cycle living in plants only in the phloem and in the hemolymph of insect species sucking in the phloem and transmitting the phytoplasmas. The Stolbur phytoplasma is occurring in South and Central Europe and the Near East and has a large host plant range, including numerous herbaceous crops such as potatoes, peppers, tomatoes, celery but also woody plants such as grapevine. Transmission of Stolbur occurs through a complicated cycle involving weed species as intermediate and principal hosts and planthoppers as vectors. The most important transmitting planthopper is *Hyalesthes obsoletus*. In Central Europe, this insect species is univoltine and overwinters as larva on roots of *Convolvulus arvensis* or *Urtica dioica*, in the Eastern Mediterranean area also on *Vitex agnus-castus*. From the roots of infected host plants the larvae take up the Stolbur phytoplasma and during larval development the phytoplasmas traverse the gut and accumulate in the salivary glands. The newly hatched adults have sufficient phytoplasma concentrations in the saliva and transmit the pathogen to healthy plants during phloem feeding. Stolbur phytoplasmas of nettle and bindweed can be distinguished based on marker genes (*tuf*, *secY*, *Stamp*) into nettle and bindweed types. The predominant type in grapevine *Bois Noir* infection in recent years is hereby the nettle type.

In 2017/18 we characterized the stolbur infections in potatoes and vegetables in Austria. The disease was detected in a wide area in large areas East and North of Vienna to the borders of the Czech Republic and Slovakia and to the West till the region of St. Pölten. Potatoes showed first signs of disease from end of June onwards. The leaves of infected plants became brittle and fragile, curled upwards and turned yellow from the shoot tip or, depending on the variety, also turned reddish. Airborne tubers sometimes formed in the leaf axils, tubers became misshaped and showed a rubbery texture. As a result, many affected plants began to wither

and die. Overall, the loss of revenue was significant. Since the insects often flew from the outside into the potato field, the disease occurred predominantly at the field margins. In addition, during the last years stolbur also caused severe damage to vegetables, such as Chinese cabbage, celery, carrots, tomatoes and peppers. In the latter two, only plants growing in open fields conditions were affected. The damage patterns caused in these plant species ranged from yellowing/reddening to severe growth changes to the death of plants. The disease was also observed in ornamental plants such as *Calendula officinalis*, *Cosmos bipinnatus*, *Tagetes erecta* and *Zinnia elegans*. Stolbur infection was confirmed by PCR and positive samples were characterized by sequencing of the marker gene *Stamp*. *Stamp* was amplified by the primers Stamp F (5'-GTAGGTTTTGGATGTTTTAAG-3') and Stamp-R0 (5'-AAATAAAGAACAAGTATAGACGA-3') and sequenced with Stamp F. In total >200 samples from the plants described above were characterized and without exception, the stolbur phytoplasma type was the bindweed type indicating that bindweed was the intermediate host and the pathogen source. Collection of *H. obsoletus* was done by vacuum sampling in the area of Ernstbrunn. Infection rates of *H. obsoletus* determined by PCR were ≈30%. In transmission experiments, insects were caged with healthy potato, tomato and *Catharanthus roseus*. Transmission of stolbur phytoplasmas of the bindweed type to the test plants was observed confirming the origin of the pathogen.

Management of stolbur is a difficult task and bindweed control aiming to reduce the pathogen source is difficult to achieve. Furthermore, infectious insects often develop on bindweeds at ruderal sites, fallows or slopes where bindweed control cannot be carried out. A successful bindweed control in fields could both reduce the sources of infection and the development of the vectors. Weed control and other possible disturbances such as mulching, vegetation mowing, herbicide use should not be carried out during the flight period of the adult insects (approximately from beginning of June to beginning of August) to keep the *H. obsoletus* in the bindweed sites. There seems to be relatively large differences in susceptibility to stolbur phytoplasma depending on the potato variety. The nature of the susceptibility can be due to tolerance to the phytoplasma, but also due to different acceptance of the in-

sects and is currently not well understood.

Currently the efficiency of insecticides for phytoplasma management is unclear. Successful management is on one hand hampered by the fact that infectious vectors move into the crop from the outside for a flight period of at least six weeks. Laboratory transmission experiments showed that successful phytoplasma transmission is accomplished with in an insect feeding time of a few hours only. In consequence fast acting insecticides with long lasting effects seem necessary. Compounds with a repellent effect such as Kaolin or diatomaceous earth could be alternative approaches. In order to monitor the effects of insecticides and repellents on insect survival and phytoplasma transmission lab experiments with *H. obsoletus* caged on differently treated *C. roseus* were carried out. A fast knockdown effect within 1-2 hours was observed for the insecticides Lambda-Cyhalothrin (Karathe Zeon, Syngenta, Austria) and Flupyradifurone (Flupyradifurone, Bayer Austria, currently no registration). Accordingly, in the case of the test plants treated with these substances, no to very few were infected with stolbur. Coatings of kaolin and diatomaceous earth had only a weak effect on the survival of the insects, but the number of infected test plants was lower than in the water control. The laboratory experiments allowed a pre selection of suitable compounds for future field experiments, which are still needed to confirm the effect of the test compounds under naturally occurring conditions.

Keywords

Bindweed · *Candidatus* Phytoplasma solani · carrot · celery · *Convolvulus arvensis* · *Hyalesthes obsoletus* · tomato

Acknowledgements

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PNYDV - still a challenge: *Pea necrotic yellow dwarf virus* in Austrian legume crops

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Abstract

The *Pea necrotic yellow dwarf virus* (PNYDV) was identified and described for the first time in Germany in 2009, and was first detected 2010 in Austria in green peas and faba beans. Hosts of the virus are mainly legumes, and aphids are reported to be the vectors. Black bean aphid (*Aphis fabae*) and pea aphid (*Acyrtosiphon pisum*) are known as major virus vectors. In 2016 and 2018, PNYDV caused severe crop losses mainly in faba beans (Fig. 1) and peas, but also in lentils and chickpeas (Fig. 2). The Austrian Agency for Health & Food Safety (AGES) is working in two projects on PNYDV. In a project supported by the Austrian Chamber of Agriculture (LK) an early warning system for the aphid virus vectors of PNYDV has been established since 2017. The national project „NANOVIR“ is dealing with the epidemiology of the virus and possibilities for aphid control in organic faba beans.

Keywords

Black bean aphid · epidemiology · nanovirus · pea aphid · *Pisum sativum* · *Vicia faba*

Early warning system for the aphid virus vectors of PNYDV

In order to be aware of an early occurrence of aphids and nanovirus infections, a warning system for aphid virus vectors in legumes was established. In 2017 and 2018, flight activity of aphids was examined on 9 different trial sites from April to May (2017)/June (2018) using yellow water traps. Aphids were tested for PNYDV by PCR analysis. In 2017, early PNYDV infections of legumes or infected aphids could not be detected, and problems caused by PNYDV in legume crops were insignificant. In 2018, anholocyclic overwintered *A. pisum* infected with PNYDV were detected on 10th April on winter field peas in the Marchfeld region. In yellow water traps, infected aphids were collected from 26th April onwards, one month earlier than in 2017. Due to the warning system, farmers were punctually informed of the occurrence of PNYDV infected aphid vectors. Consequently, targeted control measures against aphids at an early growth stage assured a harvest of legume crops.

The national project NANOVIR

PNYDV is known only for a few years, therefore, little information is available concerning the epidemiology of the virus. One of the aims of the project is to develop and collect epidemiological data.



Figure 1: Faba bean crop infected with PNYDV (Korneuburg, June 2018)



Figure 2: Chickpeas with typical symptoms for PNYDV (Grabeneegg, June 2018)

It shall be clarified, which legume species, used as either main or catch crops, are natural host plants for PNYDV, and which species are no hosts and can be recommended as catch crops. Until 2016, pea, faba bean, common vetch, grass pea and lentil were known to be hosts for PNYDV in Austria. In 2018, the first year of the NANOVIR project, we confirmed Hungarian vetch, chickpea and tiny vetch, as natural host plants for the first time in Austria (Table 1).

As Nanoviruses are transmitted by aphids, the only possibility to reduce infections is to control the aphid population. Therefore, another aim of the project is to develop recommendations for the biological control of aphids in organic faba beans.

Table 1: Summary of legume species which are host plants for PNYDV. Results are based on Ziebell (2017) with the exception of the bold printed species which was hitherto only confirmed for Austria; host species shaded in gray were confirmed for Austria).

Botanical name	Common name	German name
<i>Pisum sativum</i>	pea	Erbsen
<i>Vicia faba</i>	faba bean	Ackerbohne
<i>Vicia sativa</i>	common vetch	Futterwicke
<i>Vicia pannonica</i>	Hungarian vetch	Pannonische Wicke
<i>Lathyrus sativus</i>	grass pea	Platterbse
<i>Cicer arietinum</i>	chickpea	Kichererbse
<i>Trifolium incarnatum</i>	crimson clover	Inkarnatkleee
<i>Melilotus segetalis</i>	corn melilot	Saat-Steinklee
<i>Melilotus infestus</i>	round-fruited melilot	Steinklee
<i>Melilotus messanensis</i>	Sicilian melilot	Messina-Steinklee
<i>Melilotus italicus</i>	Italien melilot	Italiener-Steinklee
<i>Melilotus sulcatus</i>	Mediterranean melilot	Gefurchter Steinklee
<i>Lens culinaris</i>	lentil	Linsen
<i>Vicia hirsuta</i>	tiny vetch	Rauhaarige Wicke

Trials are carried out in cooperation with the company *biohelp* (www.biohelp-profi.at) to test biological solutions for aphid control. On a large trial area, two applications of 'biohelp Neudosan', an insecticidal soap based on potassium salts of fatty acids, showed a treefold higher yield than the untreated control.

In cooperation with the Agricultural Chamber of Lower Austria, the influence of intercropping field beans with oats on the occurrence of aphids will be tested. First experiences in 2018 showed that intercropping with oats inhibits the development of aphids in the faba bean crop and, therefore, reduces the incidence of PNYDV infections. While 12 *A. fabae* colonies and 169 *A. pisum* individuals were counted on 50 plants in the faba bean pure stand, only 3 and 19 individuals, respectively were found in the faba bean intercropped with oats. In the pure faba bean crop 70% of the plants showed typical symptoms of PNYDV, whereas in the intercropping stand only 30% of the plants exhibited virus symptoms.

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Micropropagation and virus elimination in elderberry (*Sambucus nigra*)

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During the last decade Styria, Austria, became the most important growth area for *Sambucus nigra* L. in Europe. About 330 farmers in the area produce 1250 hectares of elderberry. After apple, elderberry ranks second in Austrian fruit production. The harvest of berries and flowers is 6900 tons, which are nearly exclusively marketed by the Styrian soft fruit cooperative.

More than 75% of the Austrian elderberry acreage is planted with the cultivar 'Haschberg'. This leads to high costs during harvest, since most of the berries are to be harvested at the same time and need to be frozen within hours after picking.

In order to improve the spectrum of cultivars an attempt was undertaken to optimize *in vitro* propagation of five additional cultivars. Three different media were tried for establishment *in vitro*, six media were used for propagation and rooting (Table 1). A micropropagation protocol was developed using the cytokinin meta-topolin for multiplication as well as rooting of *Sambucus nigra* plants. For four out of six cultivars ('Haschberg', 'Kornberg', 'Preding', 'Blochwitz') it was possible to optimize media for micropropagation. For the cultivars 'Rubin' and 'Tatin' further work is required.

To meet the requirement for certified planting material, thermotherapy and chemotherapy (ribavirin), followed by meristem tip culture were successfully applied to eliminate various plant viruses (*i.e.* Arabis Mosaic Virus, Cherry Leafroll Virus, Strawberry Latent Ringspot Virus, Elderberry Virus A-D, Tomato Ringspot Vi-

rus, Blueberry Scorch Virus) from elderberry plants. Virus detection was carried out using RT-PCR and confirmed by electron microscopy. Using thermotherapy and meristem-tip culture, it was possible to obtain virus-free plants only from cultivar 'Haschberg'. After thermotherapy, 'Rubin' was free from all viruses tested except for Elderberry virus B. Virus elimination for cultivars 'Kornberg', 'Preding' and 'Blochwitz' could be obtained by using chemotherapy with Ribavirin (30 mg/L).

To improve the process of *ex vitro* acclimatization, plantlets rooted *in vitro* were treated with two different preparations of mycorrhiza at two different times of inoculation (*i.e.* inoculation after *in vitro* rooting before acclimatization and inoculation after 6 weeks of acclimatization in the greenhouse).

The best treatment was Mykonor Bio-Aktiv (application by dipping) inoculated after *in vitro* rooting, which reduced losses to 2% of all plants compared to 12-14% of untreated control plants. Generally, for all plants treated with mycorrhiza, regardless of the time of inoculation, a statistically significant reduction of losses and better growth (length of main shoot) was observed. Also the level of mycorrhizal colonization of roots was improved.

Keywords

In vitro propagation · mycorrhiza · virus free plant

Table 1: Culture response of six *Sambucus nigra* cultivars to multiplication (HV2m, HV3, HV4, H8P, HV5) and rooting (HV11m) media (+, good multiplication/rooting; ±, suboptimal growth; -, no growth; n.d., not determined)

Medium	Cytokinine ¹	Cultivar					
		Haschberg	Kornberg	Rubin	Preding	Blochwitz	Tatin
HV2m	BAP+TDZ	+	+	+	+	+	±
HV3	KIN	-	-	-	-	-	-
HV4	BAP	+	+	+	+	±	-
H8P	2iP	+	±	±	±	±	-
HV5	mT	+	+	±	±	±	±
HV11m	mT+NAA	+	+	n.d.	+	+	n.d.

¹ 2iP, 6-(γ,γ -dimethylallylamino)purine; BAP, N⁶ benzyladenine; KIN, kinetin; mT, meta-topolin (N⁶-(3-hydroxybenzyl)adenine); NAA, 1-napatalene acetic acid; TDZ, thidiazuron

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Winter is coming: Improving and maintaining winter hardiness and frost tolerance in bread wheat by genomic selection

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Winter hardiness is a major constraint for autumn sown crops in temperate regions, and thus an important breeding goal in the development of new bread wheat varieties. Winter hardiness is though influenced by many environmental factors and furthermore difficult to phenotype under European conditions due to the irregular occurrence of winter damage in field trials, which makes a genomic breeding strategy an interesting alternative. The aims of this study were: (i) to compare the merit of marker-assisted selection using the major frost tolerance QTL *Fr-A2* with genome-wide prediction for winter hardiness and frost tolerance; and (ii) to assess the merit of combining both measures with a genomic selection index.

A training population including 170 lines with winter hardiness data from 2012, another training population of 200 lines from a controlled frost test experiment in 2017, and an independent validation population of 130 lines that was assessed for winter hardiness in the field in 2018 were analyzed for this purpose. Cross-validation within the training population for frost tolerance showed the merit of marker-assisted selection for *Fr-A2* especially when upweighted in genome-wide prediction models, while a new QTL on chromosome 3A could be identified with the winter hardiness data from 2012. Combining both measures in a genomic selection index increased the prediction ability for the independent validation population 2018 in comparison to training with winter hardiness or frost tolerance data alone. Genomic selection showed thus high potential to improve or maintain the performance of bread wheat for this difficult and costly to phenotype trait.

Keywords

Cold tolerance · copy number variation · genomic prediction · *Triticum aestivum* · winter survival

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Molecular mapping for salinity tolerance in F₈ rice recombinant inbred lines

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Rice (*Oryza sativa*) is the staple food in Iran. However, in different parts of the country its production is affected by salinity stress. This stress suppresses the growth of rice and reduces its yield. Salinity tolerance is a complex trait and controlled by several genes. Therefore, QTL mapping is one useful tool in molecular research for studying abiotic stress.

The objectives of this study were (i) a survey of the interaction between lines for salinity tolerance in hydroponic condition, (ii) the saturation of a linkage map in a F₈ rice population derived from the cross Sepidroud × Anbarbou, and (iii) the identification of linked markers with salinity tolerance QTL

To find tolerance QTL, 96 lines of F₈ recombinant inbred lines were screened under hydroponic conditions for mapping of traits related to salinity stress and genotyped with 40 ISSR markers. The linkage map covered 1709.29 cM of the rice genome. Five QTL could be located on chromosomes 3, 5, 6, 7 and 10. The QTL qSSES-7 and qSSES-10 had the highest effects (LOD 2.99 and 3.25, respectively) and explained 27% of the phenotypic variation.

Keywords

ISSR marker · *Oryza sativa* · QTL

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Study of flowering time genes for crop improvement

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Flowering is the most crucial step that plants undergo to succeed in reproduction and survival over many generations. Therefore, it is a highly regulated process controlled by many genes interacting mutually in a genetic network and influenced by many interacting pathways and environmental influences (light, temperature). The ability to control flowering time in major crops has an impact on grain yield and thus is an attractive target for modern plant breeding efforts aiming to prepare locally well-adapted cultivars.

Bread wheat (*Triticum aestivum* L.) is a staple food for 40% population and is one of three the most important crops worldwide. Variability in the vernalization (VRN genes) and photoperiodic pathways (PPD genes) is considered the main source of plasticity and enables wheat growth in different altitudes and latitudes. Even minor variation in photoperiod and vernalization genes is associated with large differences in flowering time and thus fast adaptability of wheat to diverse locations. These changes are linked with variation at the DNA sequence level (rearrangements in the promoter region and the first intron) and also with copy number variation (CNV) of the genes. In addition, epigenetic modifications like DNA methylation and histone modifications are associated with significant changes in the flowering time determination. More investigation will be needed to fully understand this an important biological process.

Keywords

Photoperiod sensitivity · *Triticum aestivum* · vernalization

Acknowledgements

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Seedjection™ - Bringing microorganisms into seeds

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In the present time, plant beneficial microorganisms have the potential to overcome limitations we face in modern agriculture. These microbes are used as plant adjuvants or for pathogen defense but the delivery of these beneficial microbes to the plant is a major challenge. Reduction of applied cell numbers by desiccation stress or UV light are just a few of the problems of the currently used methods like seed coating or spraying. In this regard, our newly developed technology Seedjection™ (www.seedjection.at) is going a completely new way. The desired microorganisms are mechanically introduced into the seed and protected there from external influences. This mechanical approach is based on slicing the seed, injecting the desired microorganisms into the opened grain and sealing the cutting with a biodegradable sealant. Cutting the pericarp and uncovering the endosperm has no negative impact on germination, growth, plant health or shelf life of seeds. Compared to conventional application, using Seedjection™ technology, it was possible to show, for example, enhanced colonization of plant tissue with selected examples (e.g. *Bacillus amyloliquefaciens*) or enhanced storage stability of applied microbes (e.g. *Paraburkholderia phytofirmans*) as compared to seed coating. Furthermore, this technology allows accurate injection of defined volumes and due to preservation of the microbes inside of the seed, a combination of living microorganisms and an outside chemical coating is possible.

Keywords

Bacillus amyloliquefaciens · beneficial microbes · *Paraburkholderia phytofirmans* · plant-microbe interaction

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Less is better: improving forage quality of barley

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Abstract

In the framework of the Coordinated Research Project „Integrated Utilization of Cereal Mutant Varieties in Crop/Livestock Production Systems for Climate Smart Agriculture“ (2012-2018) existing mutant genetic stocks of barley were tested for their suitability to use as forage barley. The mutant genes under investigation were *Lks1* (awnless), *Kap1* (hooded lemma) and *rob1* (orange lemma). The awnless and/or hooded trait allows grazing, hay or silage production after heading, without problems of impaction of awns in the mouth and gut and damage to the eyes of cattles. The orange lemma trait shows similar phenotypic characteristics compared to the brown-midrib mutants in maize and sorghum or the red xylem mutant in poplar and results in a less acid-detergent lignin (ADL) content and higher *in vitro* organic matter digestibility.

From 2015 to 2018 more than 200 accessions were tested in field trials for grain and biomass yield and other agronomic and morphological traits. A few *Kap1*, *Lks1* and *rob1* mutant lines with both high biomass and grain yield were identified.

In vitro digestibility trials including the *rob1* mutant lines in varieties ‘Bowman’ and ‘Optic’ confirmed a significantly lower ADL content and improved organic matter digestibility as well as decreased methane formation of the mutant lines compared to their wildtype. Additionally, effects were seen on the rumen microbial community structure and the short chain fatty acids profile.

Crosses between BW666 (orange lemma mutant in ‘Bowman’) and 6-rowed hooded varieties ‘Verdant’ and ‘Sanokrihi 94’ were carried out to combine both mutant genes of interest. As most genes in the lignin biosynthesis pathway have been well characterized, a candidate gene approach (HvCAD2) was used to develop a func-

ional marker for the monofactorial recessive *rob1* gene. Candidate causative mutations underlying the orange lemma phenotype were identified and an allele-specific assay was developed for *rob1* in the ‘Bowman’ genetic background. Using a segregating F₂ population, trait and marker linkage could be demonstrated. Experiments have been initiated to validate the marker assay in additional barley genetic backgrounds to enable marker-assisted backcrossing of *rob1* for forage barley improvement.

Keywords

Digestibility · forage · *Hordeum vulgare* · lignin · livestock · marker-assisted selection · mutation breeding

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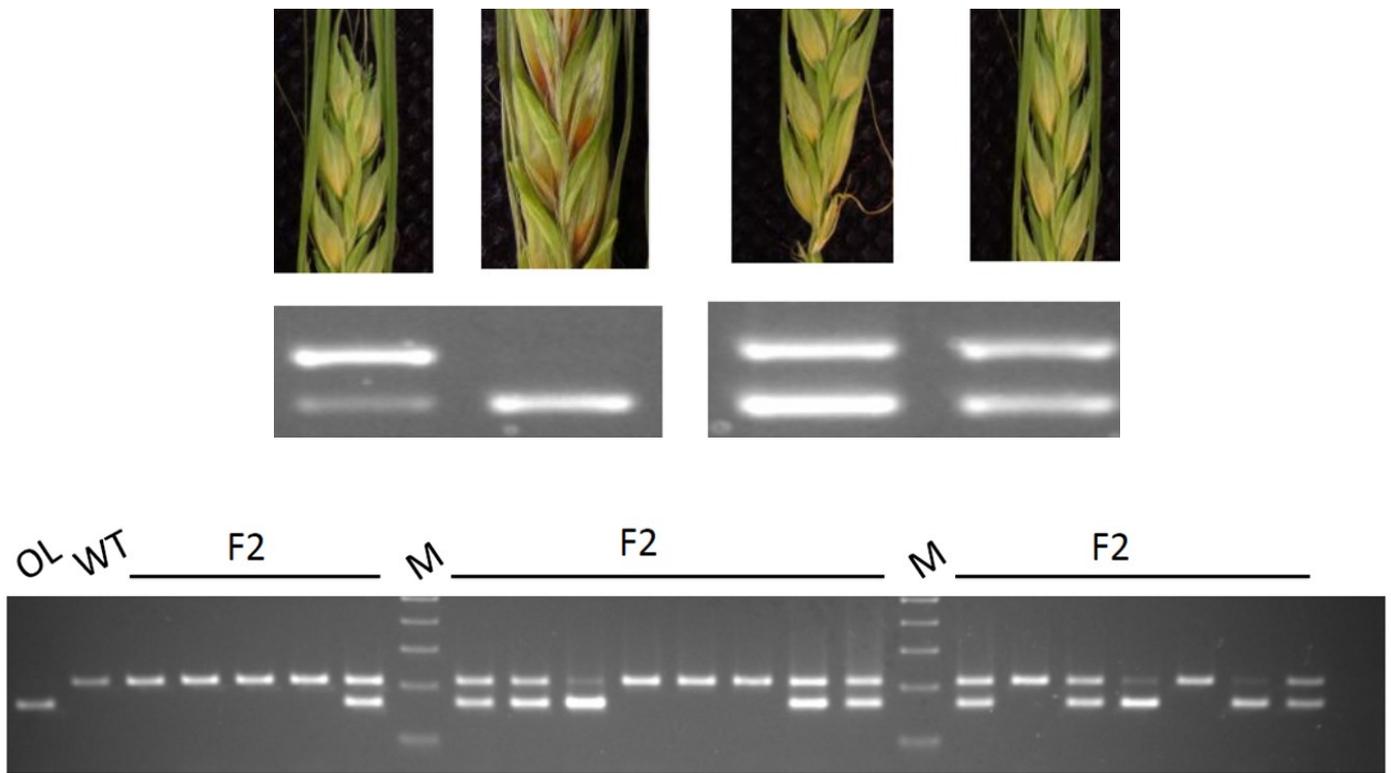


Figure 1: Allele specific marker development for the orange lemma mutant gene: phenotype and marker expression of wildtype (WT) and orange lemma (OL) parental lines and heterozygous F₁ plants (top from left to right), and marker expression in a segregating F₂ population (bottom).

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Soybean breeding for organic farming: breeding goals and options

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Abstract

Domestic soybean production has increased in many countries of Central Europe over the last decade. As a significant share of soybean production is under organic management at present, a growing demand for varieties suitable for organic production systems has developed. For this reason, the specific needs of cultivars in organic production and possible breeding options are elaborated. Apart from grain yield, weed suppression/tolerance is a highly desirable trait that could be determined in inter-seeding experiments for simulating weed pressure. Other traits such as resistance against diseases and pests as well as tolerance to abiotic stresses (chilling, high temperature and drought) can be selected similarly in conventional and organic programs. In contrast, efficient selection for symbiotic di-nitrogen fixation would require dedicated approaches such as continuous selection under low-nitrogen soil conditions. As individual organic breeding activities are small, collaborative approaches between plant breeding organisations on a European level could increase the efficiency of breeding work.

Keywords

Biological di-nitrogen fixation · *Glycine max* · image analysis · weed suppression

Introduction

In recent years, domestic production of soybean (*Glycine max* [L.] Merr.) has gained importance in many European countries. Within one decade, the total European soybean acreage has increased from 1.6 mio ha (2008) to over 5.7 mio ha (2017) according to FAO figures (FAO-STAT 2019). In Austria, soybean cropping has peaked in 2018 with an acreage of 67 500 ha, and almost 20 000 ha (29%) of that area have been planted under organic management (AMA 2018). Among farmers, soybean production is popular due to its favorable economic competitiveness against other crop species. For Austria, an average yield increase of +30.2 kg per ha per year has been calculated for the period of the past 30 years (1988-2017) of soybean growing which represents a major plant breeding

achievement. Moreover, a recent analysis of crop yields for conventional vs. organic farming across Austria from 2003 to 2016 has revealed that organic farming yields dropped to 60-70% of conventional yields for most cereal species and to about 50% for potatoes, whereas soybean organic yields were in the range from 93-109% of conventional yields (Brückler *et al.* 2017). This finding explains the particular attractiveness of soybean cropping in organic farming.

At present, there are over 60 soybean cultivars from maturity groups 0000 to 0 registered in Austria, but only a few of them are utilized in the organic farming sector. As there is a growing need for certified organic seed and for regionally developed and well-adapted cultivars particularly suitable for organic management, organic breeding and/or breeding and selection steps under organic growing conditions are gaining in importance. While agronomic and phenology traits such as grain yield, environmental stress tolerance or time to maturity are relevant to soybean production irrespective of the farming system, other characteristics such as competitiveness against weeds, symbiotic di-nitrogen fixation and seed quality features are typically more important for cultivars to be grown under organic management. Thus, as organic farming resembles a specific set of environmental conditions characterized by low rates of mineralized soil nitrogen, a highly diverse soil microbiome and stronger weed pressure, adaptation to such conditions appears as a prerequisite for successful performance. For this reason, the specific needs and options of soybean breeding for organic farming conditions are highlighted and discussed in the light of previous results. Furthermore, an outlook is given to an initiative for increasing efficiency and competitiveness of organic plant breeding.

Specific needs and breeding options

Grain yield

Grain yield is the parameter ensuring competitiveness of a crop on the individual farm level as well as on the level of crop rotations. While yield progress (30.2 kg per ha per year for Austria, comparable progress in other European countries as well) appears sufficient at present, this progress might slow down in the future. On a



Figure 1: Difference in early ground cover at the pre-flowering stage between two soybean breeding lines with ovate (left) or narrow (right) leaflet shape.

world wide scale, a majority of 77% of the soybean acreage has been planted with GMO soybeans in 2017 (Forum Bio- und Gentechnologie eV 2019), but GMO soybeans cannot be utilized for non-GMO and organic breeding (Messmer *et al.* 2015) thus probably reducing future breeding progress for yield and competitiveness because of the limited number of remaining non-GMO programs and reduced options for exchange of elite breeding materials.

Competitiveness against weeds

Weed control is a major issue both in conventional and organic soybean production in Europe. Efficient weed management strategies have been established for organic soybean production based on delayed sowing, wide-row-seeding and several rounds of mechanical weed control (Bernet *et al.* 2016; Djordjević *et al.* 2016). Nevertheless, competitiveness of soybeans against weeds is a highly desirable trait as weed infestation is reducing both yield and quality of the harvest (Carkner *et al.* 2017; Vollmann & Menken 2012). Soybean weed competitiveness is mediated by early vigor and ground cover for suppressing weed biomass development as well as by tolerance to weeds at later stages of development. Genetic differences in early vigor have been described, and their relation to sustained later development has been discussed (Jannink *et al.* 2001). Screening for early development could be carried out by scoring for plant biomass development at critical stages, but canopy coverage could most precisely be determined by overhead photography with subsequent digital image analysis or by measuring light interception (*e.g.* Place *et al.* 2011). An example for differences in early ground cover between soybean genotypes is given in Fig. 1.

Selection for weed competitiveness based on natural weed occurrence is difficult in small plot experiments due to spatial variation in weed distribution. Therefore, sowing of weed seeds or crops for simulation of a homogeneous weed pressure has been practiced. Hand-sowing of winter rapeseed into soybean plots three to four weeks after soybean planting produced a significant weed pressure throughout the soybean growing season (Fig. 2). As compared to weed free plots, soybean yields were reduced by 15-30% under weed infestation in two seasons, but higher yields under weed infestation were found in another season with higher precipitation rate at a later stage (Vollmann *et al.* 2010). Time to maturity, plant height, 1000-seed weight as well as oil content were also affected by the weed treatment. Genotype differences in

yield losses were consistent across growing seasons (Fig. 3) suggesting the presence of a reproducible genetic variation.

Instead of inter-seeding winter rapeseed only, Horneburg *et al.* (2017) proposed a seed mixture of ten different species to better simulate natural weed infestation and weed competition in soybean plots. They observed yield reductions from 20 to 60% as compared to weed-free controls but did not find differences in weed tolerance among six early maturity soybean genotypes. In general, the results demonstrate the experimental feasibility of a direct selection system for weed tolerance based on sowing competitors into soybean plots.

Symbiotic di-nitrogen fixation

Symbiotic di-nitrogen fixation by rhizobial bacteria is the relevant biological process ensuring favorable yield performance and harvest product quality (Zimmer *et al.* 2016). Additionally, in organic farming, legume cropping has an important function on the nitrogen balance of a crop rotation. Soybean is known to be able to fix around 80 to 120 kg/ha nitrogen per season. However, based on the assumption of an average soybean grain yield of 3000 kg/ha and a seed protein content of 40% based on dry matter, over 190 kg/ha nitrogen are exported from the field through a crop harvest. As a consequence, nitrogen balance of soybean can be negative particularly in environments with high soybean yield (Salvagiotti *et al.* 2008). This is supported by findings from Central European environments demonstrating that a rate of only 40-57% of the soybean nitrogen uptake is from biological nitrogen fixation (Schweiger *et al.* 2012; Zimmer *et al.* 2016). Therefore, selection for higher di-nitrogen fixation is of interest to improve both crop performance and nitrogen balance for subsequently enhancing the crop rotation value of soybean. As a direct measurement of di-nitrogen fixation using N-isotope-based methods such as the ¹⁵N natural abundance method is laborious (Schweiger *et al.* 2012) and not suitable for screening large numbers of breeding lines, there is a need for the implementation of indirect screening methods. Selection for nitrogen yield (derived from grain yield and protein content) illustrates the nitrogen uptake, but cannot differentiate between soil uptake and atmospheric nitrogen fixation. This also applies to other phenotyping methods such as chlorophyll metering, leaf image analysis or hyperspectral reflectance measurements (*e.g.* Vollmann *et al.* 2011), which can comparatively determine the nitrogen status of breeding lines. Thus, selection under conditions of low-mineralized-nitrogen content of soil



Figure 2: Simulated weed pressure (winter rapeseed sown into soybean plots) at flowering (top row) and maturity (bottom row) stages of soybean development.

(i.e. organic soil conditions) is the method of choice, as it would best reflect biological nitrogen fixation capacity and could contribute to identify soybean genotypes with improved nitrogen balance. Moreover, in the long-term perspective, selection under nitrogen-depleted soil conditions would better allow to identify rhizobacterial strains efficient in nitrogen fixation (Chen *et al.* 2015) and elucidate the existence of specific soybean genotype by rhizobial strain interactions (Zimmer *et al.* 2016). In addition, the evolution of efficient rhizobial strains (Weese *et al.* 2015) as well as the soybean genotype capability to recognize efficient bacterial strains both rely on low-nitrogen soil conditions (Kiers *et al.* 2007). This all advocates for selection under organic/low-input soil and environmental conditions in order to optimize symbiotic dinitrogen fixation.

Stress tolerance

Soybean is exposed to different types of abiotic stress depending on regional and seasonal conditions. In Central Europe, chilling tolerance at early developmental stages and at flowering time is important, whereas in Southern European environments, tolerance to high temperature and drought are most relevant. All of these types of abiotic stress tolerance are based on quantitative inheritance and require appropriate screening which applies to breeding for organic and conventional farming as well. Better adaptation to specific environmental conditions as mediated by progress in classifying soybean genotypes in terms of flowering/maturity genes (Miladinović *et al.* 2018) might also contribute to mitigate abiotic stress.

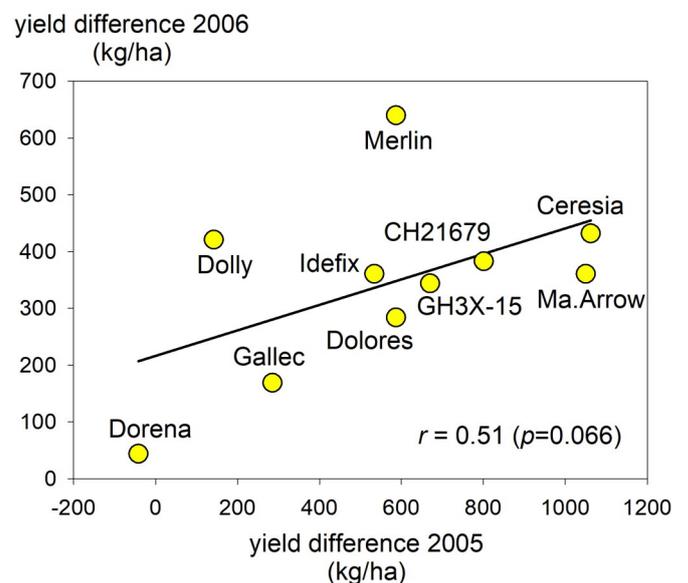


Figure 3: Soybean yield loss (yield difference between weed-free and weed-pressure plots) for ten soybean genotypes across two seasons.

Harvest product quality

Significant proportions of the organic soybean harvest are utilized in food manufacturing. Thus, food-grade soybean quality characters centering on seed protein content, protein properties and soy health features might be of interest to soybean breeding (Vollmann & Menken 2012). In addition, the integration of food safety traits (Watanabe *et al.* 2018) would strengthen the position of organic soybean as a preferable raw material for soy-food production.

Genetic diversity needs and restrictions

Although a remarkable genetic diversity is preserved in soybean germplasm collections (Carter *et al.* 2004), the diversity utilized in breeding programs is rather small due to the need for crossing within 1-2 maturity groups only, a narrow genetic diversity in early maturity germplasm, and the limited number of GMO-free breeding programs at present. Thus, marker-assisted introgression of novel diversity and traits into elite breeding material of early maturity is an important task for maintaining diversity and yield progress.

Outlook to ECOBREED experiments

Individual activities in organic soybean breeding are small at present. Therefore, collaborative efforts between plant breeding institutions on a European level could increase the efficiency of cultivar development leading to significant innovations in organic soybean production. The Horizon 2020 project ECOBREED is focusing on increasing the availability of seed and varieties for the organic farming and low-input sector. In soybean, ECOBREED activities will include field trials with elite germplasm and genetic resources focusing on yield, competitiveness against weeds, tolerance to selected diseases and pests, grain quality, and chilling and drought tolerance. In addition, breeding materials will be genotyped, screening methods for nitrogen fixation will be elaborated, and seed multiplication experiments will be carried out utilizing cover crops and seed inoculants.

Conclusions

A growing demand for soybean cultivars suitable for organic farming is prompting for dedicated breeding activities to better meet the specific requirements of the organic sector. Characters such as weed tolerance/suppression or tolerance to abiotic and biotic stress factors could probably be covered in conventional programs with specific testing in organic farming environments, whereas the long-term goal of an improved di-nitrogen fixation would require a complete selection program under low-nitrogen conditions to get the full benefit of rhizobial symbiosis.

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ECOBREED – Increasing the Efficiency and COMPETITIVENESS of organic crop BREEDing. A new H2020 project on organic breeding of wheat, potato, soybean and buckwheat

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Abstract

The Horizon 2020 project ECOBREED (www.ecobreed.eu) is coordinated by the Agricultural Institute of Slovenia and is carried out in collaboration with 25 partner organisations from 15 countries, *i.e.* Austria, China, Czech Republic, Germany, Greece, Italy, Poland, Spain, Hungary, Romania, Serbia, Slovakia, Slovenia, United Kingdom and the United States. ECOBREED will improve the availability of seed and varieties suitable for organic and low-input production. The activities will focus on four crop species, *i.e.* wheat (*Triticum aestivum* and *T. durum*), potato (*Solanum tuberosum*), soybean (*Glycine max*) and common buckwheat (*Fagopyrum esculentum*). Objectives of the project are among others the increase of breeding activities for organic and low-input crop production and the development of breeding material with improved stress resistance, resource use efficiency and quality.

In wheat, the focus lies on the development of bunt (*Tilletia caries*, *T. controversa*) resistant genotypes by marker-assisted selection (MAS), and the development of new 'populations' including genetic material from all involved partners following a multiparent advanced generation intercross scheme. Moreover, genotypic effects will be evaluated with respect to arbuscular mycorrhizal colonisation and *in vitro* root exudation of allelochemicals.

MAS will also be exploited in potato to select advanced clones with resistance against potato virus Y (PVY) and late blight (*Phytophthora infestans*) using the 'Sarpò Mira' genepool and other resistant germplasm. Moreover, different strategies for a sustainable control of Colorado potato beetle and wireworms will be evaluated under field conditions.

Soybean will be tested for the competitiveness against weeds and for tolerance to naturally occurring biotic stresses and abiotic stress factors such as drought and chilling. MAS will be carried out for the identification of germplasm with low cadmium accumulation in seeds, supernodulation and drought tolerant biological nitrogen fixation, and resistance against *Sclerotinia sclerotiorum* and the *Diaporthe* complex.

Buckwheat genetic resources and breeding material will be screened for allelopathic activity and for genetic variation in phosphorus mineralisation. To identify the underlying genetic responses to abiotic (cold, drought, salinity) and biotic stresses together with quality traits, the germplasm will be genotyped by sequencing.

The specific tasks within the individual crops will be accompanied by the screening of a broad range of genetic material in all four crops in organic multi-environment trials. Based on these and previous results, new crossings among suitable parental genotypes will be carried out and distributed among partners for further selection. Thereby, ECOBREED will enhance the portfolio of wheat, potato, soybean and buckwheat varieties suitable for organic farming in Europe and identify traits and combinations of traits suited to organic and low-input farming.

Besides the breeding activities, farmer participatory trials will be established in different ecogeographical regions and training courses on *e.g.* advanced genotyping and phenotyping methods, and participatory plant breeding will be organised. Demonstration, testing and training activities within ECOBREED, in particular in EU Member States where the organic sector is less developed will help to fulfil this gap.



Figure 1: Wheat crosses at Selgen a.s., Stupice, Czech Republic, organic wheat variety testing near Murska Sobota, Slovenia, and potato clone multiplication and crosses at the Agricultural Institute of Slovenia, Infrastructure Center Jablje (clockwise from top left).

Keywords

Buckwheat · disease resistance · marker-assisted selection · organic plant breeding · potato · soybean · wheat

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Descriptive and recommended variety lists in European countries and their scales

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Abstract

In many European countries, institutes or authorities publish descriptive or recommended lists of varieties, or trial reports which provide information about the performance of agricultural plant varieties. Descriptive variety lists are published in Austria, Denmark, Luxembourg, Slovenia and Spain. Recommended lists of varieties are published in Ireland, the Netherlands and Switzerland. Institutions in Belgium, the Czech Republic, France, Germany, Poland and the United Kingdom publish both descriptive and recommended lists of varieties. In other countries, trial reports are published, which contain the results from one or more years of testing. Plant height and quality characteristics are often presented in the form of absolute values whilst yields are usually quoted relative to standard varieties. A scale that ranges from 1 to 9 is most commonly used to characterise agronomic variety traits. Different scales are used between countries, such as those that range from 0 to 9 (Spain), 2 to 9 (Netherlands), 1 to 10 (Norway), 0 to 100 (Sweden), ++++ to - - - (Switzerland) and +++ to - - - (Bavaria). Sometimes a verbal scale (*e.g.*, resistant, susceptible) is used that has two to seven categories. However, the directions of the scales are not always the same; endpoints are either marked as 'very low to very strong' or 'very favourable to very unfavourable'. Diseases may be described using the opposing terms 'susceptibility to' or 'resistance to'. In Europe, there is a pronounced heterogeneity in the descriptions of valuable variety characteristics.

Keywords

Description · post-registration trials · recommendation · variety list · VCU testing

Introduction

New plant varieties are subject to statutory registration through official VCU (Value for Cultivation and Use) trials before they can be marketed in the EU. In order to define the characteristics of the varieties, field trials are carried out in important cultivation areas for the respective species and the quality of the harvested product is assessed. Many European countries also publish recommended or descriptive variety lists, whereby the aim is to objectively describe registered and commercially available plant varieties in terms of their yield potential, agronomic and quality traits, and resistance to pests and diseases. Given the diversity of varieties

available on the market, these publications provide information that is valuable to farmers, breeders, seed companies, crop consultants, agricultural traders, processors, and other users throughout the agricultural industry.

Variety lists and trial reports

It has not yet been clearly defined how Descriptive Lists of varieties, Recommended Lists of varieties and Trial Reports should be distinguished from each other in terms of their content. In this paper, we use the name that is used by the institution which has published the material.

The **Descriptive Lists of varieties** contain all registered varieties of agricultural plant species (other than conservation varieties and varieties with a much older registration date) or a selection thereof. Sometimes varieties that have not been registered in the country concerned (EU varieties) are included. Descriptive lists of varieties provide a quick, comparative overview of the varieties, their characteristics, and value. Such lists exist, for example, in Austria, Belgium, Denmark, France, Germany, Poland and Spain.

A **Recommended List** usually contains a clearly limited set of varieties. The results are based on an extended network of field trials and have a higher level of detail, thus providing the industry with more information. The institution or expert panels decide which varieties are included in trial for a recommended list. In some instances, the application must be filed by the breeder or variety representative. Recommended variety lists are available in the United Kingdom, the Czech Republic, France, Germany, Ireland, the Netherlands, Poland, and Switzerland. In some German federal states and in Poland, the regional recommendation is limited to naming the variety denomination and the specific characteristics can be found in the descriptive list of the crop.

In Lithuania and Romania, the **National List** contains not only administrative data (*e.g.*, registration date, applicant, maintainer), but also information about the valuable characteristics of newly registered varieties which can be found in a separate chapter.

In a number of countries (Bulgaria, Croatia, Estonia, Finland, Hungary, Latvia, Lithuania, Norway, Romania, Serbia, Slovakia, Sweden), there is no descriptive or recommended list of varieties in the strict sense. The public receives information about the value of the varieties through **Trial Reports**. Sometimes, the results from several years of trials are summarised and the information is similar to that which appears in a descriptive list of varieties.

Description by states of expression

Varieties are characterised by a number of traits, such as plant height, ripening time, lodging, tendency toward pre-harvest germination, susceptibility to diseases and pests, yield potential and quality characteristics. To make the documents easier to read and eliminate the influence of different test periods, the measured values are often converted into states of expression. These are calculated using average values from orthogonal datasets and adjusted average values on the basis of differences between the tested varieties and reference varieties that have been tested over many years. A scale that ranges from 1 to 9 is predominantly used, but the direction of the scale differs. Averaged and untransformed scoring values are sometimes used (Table 1). Instead of numbers, signatures (+, 0, -) or letters are also sometimes used. Verbalised scales are often used for potatoes.

Furthermore, conflicting terms are in use for certain features. In particular, this applies to agronomic properties such as the sensitivity to winter kill, brackling, lodging, necking, grain shedding and pre-harvest germination. The opposing terms of ‘resistance to’ these characteristics is also used. The use of the phrases ‘resistance to’ instead of a ‘tendency toward’ or ‘susceptibility to’ can require different scoring methods to obtain the same result with a scale that ranges from ‘very low’ to ‘very strong’.

Descriptive or recommended lists of varieties contain data collected over two to more than ten years. In terms of diseases, and especially mildew and rust fungi, new races may change the resistance of the varieties to the particular disease. In such cases, results that have been collected over short periods (one to three years) are used for these classifications. Separate assessments are sometimes made if disease data are available from natural environments or inoculated trials.

Table 1: Characterization of Variety Characteristics in Descriptive Lists, Recommended Lists and Trial Reports of European Countries

Country	List ¹	Diseases ²	Presentation, description and scales
Austria (until Oct 2018)	DL	S	Absolute values, relative yield, differences, 1 = very favourable (<i>e.g.</i> very low susceptibility, very high yield), 9 = very unfavourable (<i>e.g.</i> very high susceptibility, very low yield), verbal scales
Austria (since Nov 2018)	DL	S	Absolute values, relative yield, differences, 1 = very low degree (<i>e.g.</i> very low susceptibility, very low yield), 9 = very high degree (<i>e.g.</i> very high susceptibility, very high yield), verbal scales
United Kingdom	DL, RL	R	Absolute values, relative yield, differences, %-scale (lodging), 1 = very low degree (<i>e.g.</i> very low resistance), 9 = very high degree (<i>e.g.</i> very high resistance)
Belgium	DL, RL	R	Absolute values, relative values (plant height, yield), 1 = very low degree (<i>e.g.</i> very low resistance), 9 = very high degree (<i>e.g.</i> very high resistance)
Bulgaria	TR	I	Absolute values, 1 = very unfavourable, 9 = very favourable
Croatia	TR	I	Absolute values, %-scale (lodging), 1 = very favourable (<i>e.g.</i> no lodging, no disease infestation), 9 = very unfavourable (<i>e.g.</i> very heavy lodging, very severe disease infestation)
Czech Republic	DL, RL	R	Absolute values, relative yield, differences, 1 = very low degree (<i>e.g.</i> very low resistance), 9 = very high degree (<i>e.g.</i> very high resistance), signatures of +, 0, - (falling number stability)
Denmark	DL	S	Absolute values, relative yield, 1 = low degree (<i>e.g.</i> very low susceptibility), 9 = very high degree (<i>e.g.</i> very high susceptibility)
Estonia	TR	I	Absolute values, 1 = very favourable (<i>e.g.</i> no lodging, no disease infestation), 9 = very unfavourable (<i>e.g.</i> very heavy lodging, very severe disease infestation), reversed scale on winter damages
Finland	TR	I	Absolute values, relative yield, %-scale (winter damages, lodging, disease infestation)
France	DL, RL	R, S	Absolute values, relative yield, differences, %-scale (some diseases), 1 = very unfavourable (<i>e.g.</i> very low resistance), 9 = very favourable (<i>e.g.</i> very high resistance), four-step and six-step verbally scales
Germany	DL	S	1 = very low degree (<i>e.g.</i> very low susceptibility), 9 = very high degree (<i>e.g.</i> very high susceptibility), signatures of ++ to -- (falling number stability), verbal scales (partly with potatoes)
Bavaria	DL, RL	R	Absolute values, relative yield, +++ (very good, very high, very early, very short) to --- (very bad, very low, very late, very tall), for organic farming reversed scale on plant height (+++ = very tall, --- = very short), verbal scales
Hesse, North Rhine-Westphalia	DL, RL	S	Absolute values, relative yield, 1 = very low degree (<i>e.g.</i> very low susceptibility), 9 = very high degree (<i>e.g.</i> very high susceptibility)
Further German Federal States	DL, RL	R, (S)	Absolute values, relative yield, +++ (very good, very high, very short, or above average) to --- (very bad, very low, very tall, or below average), for organic farming reversed scale on plant height (+++ = very tall, --- = very short), 1 = very low degree, 9 = very high degree, verbal scales

Table 1: continued

Country	List ¹	Diseases ²	Presentation, description and scales
Hungary	TR	I	Absolute values, relative yield, differences, %-scale, 1 = very unfavourable (e.g. very severe disease infestation), 9 = very favourable (e.g. no disease infestation)
Ireland	(DL), RL	R	Absolute values, relative yield, 1 = very unfavourable (e.g. very low resistance), 9 = very favourable (e.g. very high resistance)
Latvia	TR	R	Absolute values, relative yield, %-scale (some diseases), 1 = very unfavourable (e.g. very low resistance, very low quality), 9 = very favourable (e.g. very high resistance, very high quality)
Lithuania	TR	R	Absolute values, 1 = very unfavourable (e.g. very low resistance), 9 = very favourable (e.g. very high resistance)
Luxembourg	DL	R, S	1 = very low degree (e.g. very low resistance), 9 = very high degree (e.g. very high resistance), on diseases of potatoes 1 = very low susceptibility, 9 = very high susceptibility, verbal scales
The Netherlands	RL	R	Absolute values, relative values (plant height, yield), 2 = very unfavourable (e.g. very low resistance), 9 = very favourable (e.g. very high resistance)
Norway	TR	R	Absolute values, relative yield, differences, %-scale, 1 = very high growth, very low resistance, very low quality, 10 = very short growth, very high resistance, very high quality
Poland	DL, RL	R	Absolute values, relative yield, 1 = very unfavourable (e.g. very low resistance, very low quality), 9 = very favourable (e.g. very high resistance, very high quality)
Romania	TR	I	Absolute values, 1 = very favourable (e.g. no disease infestation), 9 = very unfavourable (e.g. very severe disease infestation), reversed scale on turf grasses (1 = very weak, 9 = very favourable)
Serbia	TR	I	Absolute values, 1 = very favourable (e.g. no lodging), 9 = very unfavourable (e.g. very heavy lodging), reversed scale on legumes and forage crops
Slovakia	TR	S	Absolute values, 1 = very unfavourable (e.g. very high susceptibility), 9 = very favourable (e.g. resistant)
Slovenia	DL, TR	I, R, S	Relative yield, 1 = very favourable (e.g., no disease infestation), 9 = very unfavourable (e.g. very severe disease infestation), also reversed scale (1 = very high susceptibility, 9 = very low susceptibility), further scales with 4 to 10 steps, +++ (very good, very high) to --- (very bad, very low), verbal scales
Spain	DL, TR	I, R	Absolute values, relative yield, %-scale (lodging, diseases), 0 = no disease infestation, 9 = very severe disease infestation, verbal scales
Sweden	TR	I	Absolute values, relative yield, %-scale (winter damages, lodging, diseases)
Switzerland	RL	R	Absolute values, ++++ = excellent, +++ = very good to --- = very poor, on forage crops 1 = favourable (e.g. very high speed of development in spring, very high yield, very high resistance to diseases) to 9 = unfavourable, verbal scales

¹ DL = Descriptive List, RL = Recommended List, TR = Trial Report

² I = Infestation, R = Resistance to, S = Susceptibility to

Austria

Development of the Descriptive List of Varieties

The Seed Act of 1937 (*Saatgutgesetz* 1937, Federal Law Gazette No. 236/1937), Plant Breeding Act of 1946 (*Pflanzenzuchtgesetz* 1946, No. 34/1947) and the Seed Law Amendment of 1964 (*Saatgutgesetznovelle* 1964, No. 195/1964) did not explicitly require the variety traits to be systematically published. Nevertheless, a description of agricultural crop varieties (Bauer 1961, Bundesanstalt 1962) was published early on and nine supplements to this publication were issued up until 1970. The new editions (Bundesanstalt 1971, 1986) were issued in the form of ring binders with insert sheets. One disadvantage of these variety descriptions was the fixed rating used. Additional results or altered susceptibility to a disease could only be communicated in a new edition.

Therefore, an annual summary of results from many years of trials was published (Bundesanstalt 1965-1994, BFL 1995-1996). The

states of expression, which ranged from 1 to 9, were regularly adjusted and current yield results were published in this summary.

Plant varieties are registered by the Federal Office for Food Safety (BAES) after undergoing two or three years of trials. The Austrian Agency for Health and Food Safety (AGES) is responsible for conducting these experimental trials. The VCU trials involve breeders, seed companies, and other stakeholders. The Seed Act of 1997 (*Saatgutgesetz* 1997, No. 72/1997, § 65 (3)) states that the characteristics of the varieties that are essential for their cultivation and suitability under certain soil and climatic conditions need to be included in a descriptive list of varieties (§ 65 (3)). The Descriptive List of Varieties (BFL 1997-2002, AGES 2003-2019) is updated and published annually. It contains information on almost all varieties of cereals, maize, sorghum, grain legumes, oil plants, beets, potato, forage crops, and catch crops that have been approved in Austria. It contains several years of the results from official VCU trials and other variety trials. Regional yield performance results are presented for some plant species and a limited

set (of varieties). As a result, the Austrian descriptive list of varieties also contains information that appears in a recommended list.

Former meaning of the states of expression

The descriptions of agricultural crop varieties (Bundesanstalt 1962) included descriptions of valuable characteristics, assessed on the basis of a nine-step word scale. The following edition (Bundesanstalt 1971) and the summary of experimental results from multiple years that was issued from 1972 and on used a numeric scale. On this scale, the rating of 1 was the most economically favourable and the rating of 9 was the most unfavourable characteristic of the feature. A variety that matured very early and displayed very short height received a rating of 1 (Hron 1976). However, the associated verbal meaning has changed over time. For example, a rating of 2 initially meant that the variety displayed early maturity, short height, and a high yield, and a rating of 8 indicated that the variety displayed late maturity, tall height, and a low yield. In later years, however, respective ratings of 3 and 7 were awarded for these characteristics. Furthermore, composite assessments such as 'high to medium' and 'medium to high' (assigned ratings of 3 and 4, respectively, regarding yield) and 'strong to medium' and 'medium to strong' (assigned ratings of 7 and 6, respectively, regarding susceptibility to diseases) had different meanings up until 1998.

Limitations of the previous states of expression

The rating of '1 = very favourable, 9 = very unfavourable' was easy to apply when assessing variety characteristics such as winter kill, brackling/lodging, stem breakage, diseases, yield, hectoliter weights, or flour yield and so on. In these cases, there was no doubt about what was positive or negative. With respect to other characteristics, however, this scheme could be applied less effectively or only under certain conditions. For example, a short height was termed 'generally favourable'. With soybean, however, this feature can contribute to more grain loss. Current spring durum wheat and spring barley varieties consistently have quite short stems. A spring barley variety that is given a plant height rating of 2 (very short to short) shows no advantage over a variety with a height rating of 4 (short to medium). Medium- or long-stemmed cereal varieties are more suitable for cultivation under organic conditions or on farms which need the straw. Silage maize, rye for fodder purposes, or triticale for biogas use that display slightly longer stem-growth is even favourable. Early maturity, indicated by a low rating, is not as valuable as low susceptibility to disease, because the growing season in autumn can be exploited for maize, soybean, sunflower, or potato to obtain extra yield. A higher protein content is advantageous in bread wheat, durum wheat, feed barley, and feed triticale, but brewing cereals or wheat for producing starch or biscuits should contain less protein. Rye varieties with very high falling numbers and amylogram values maintain their quality longer in weather that promotes pre-harvest germination and are popular with farmers. However, rye varieties with a falling number of 150 to 200 s and a viscosity of 600 to 800 amylogram units (AU) are optimal for baking. Millers and bakers are dissatisfied with the values of more than 300 s and more than 1500 AU, respectively, which frequently occur when the variety ripens

under dry conditions. Doughs from baking wheat varieties should bind an amount of water that is above average and remain stable during kneading. In contrast, a wheat variety used to make biscuits requires a low capacity of absorb water and low stability during kneading. Extremes values of dough extensibility and resistance to extension are usually undesirable and, instead, these values should be optimal balanced. If a malting barley had a very high Kolbach number, it was given a rating of 1 (generally favourable). However, most master brewers now prefer to have a medium-high amount of nitrogen in the wort (*i.e.*, 39 to 42%). Potato varieties for food need the highest possible number of medium-sized tubers, but starch potatoes can be large. An early-season potato should not be negatively assessed for having a low starch content.

New scheme

One can avoid these difficulties by avoiding the phrases 'generally favourable' or 'generally unfavourable' in variety descriptions. In November 2018, the rule was changed to '1 = to a very low degree, 9 = to a very high degree' (Table 2). The new scheme describes the varieties, but also allows users of the variety list to make judgements according to their own requirements (Oberforster 2019). Depending on the characteristic or use of the variety, a degree that is assessed as low (low rating) may be favourable or unfavourable, and the same consideration applies to a degree that is assessed as strong (high rating). Regarding yield traits, nitrogen efficiency and many quality characteristics, this requires altering the scale direction. About half of the characteristics of common wheat, durum wheat, and malting barley are now assessed using the opposite scale. In the case of maize, the characteristics of early development, grain yield and dry matter yield, and proportion of cobs are affected. Early development, grain yield, thousand grain weight, and oil content of sunflower and oil pumpkin, as well as the protein content of soybean, are now scaled differently. The direction of the scale has had to be adjusted for individual characteristics in other plant species as well.

Table 2: Austrian 2019 descriptive list of varieties for winter wheat (selected varieties and traits). Scale: 1 = very low degree (very early, very short, very low tendency toward lodging, very low susceptibility to diseases etc.); 9 = very high degree (very late, very tall, very high tendency toward lodging, very strong susceptibility to diseases etc.)

Variety	tendency toward, susceptibility to							Quality traits											
	Maturity	Plant height	Lodging	Sprouting	Powdery mildew	Leaf rust	Yellow rust	Fusarium head blight	Grain yield, Pannonian region	Grain yield, Humid region	Thousand grain weight	Test weight	Crude protein content	Falling number	Water absorption of dough	Farinograph quality number	Dough energy	Baking volume	Baking quality group
<i>High quality wheat</i>																			
Arnold	2	6	5	4	4	5	5	4	3	3	6	9	9	5	9	8	8	8	8
Aurelius	4	4	3	2	3	4	3	6	7	6	6	8	5	7	4	7	9	7	7
Bernstein	7	6	3	4	6	7	1	4	7	6	7	7	6	7	6	7	8	8	8
Energó	4	6	4	3	3	6	4	4	5	5	6	7	6	5	8	6	7	7	7
<i>Bread wheat</i>																			
Siegfried	7	4	3	5	3	6	2	4	8	9	6	5	3	6	4	3	4	4	4
Spontan	5	4	2	6	3	6	2	4	-	8	6	6	5	5	8	5	6	4	4

Table 3: United Kingdom – AHDB (2018) Recommended List, winter wheat 2019/20 (selected varieties and traits). On the 1 to 9 scales, high figures indicate that a variety shows the character to a high degree (e.g. high resistance). Comparisons of varieties across regions are not valid. [], limited data; @, believed to carry the *Pch1* Rendezvous resistance gene to eyespot but this has not been verified in Recommended List tests; R, believed to be resistant to orange wheat blossom midge (OWBM) but this has not been verified in Recommended List tests.

	KWS Zyatt	Skyfall	KWS Trinity	RGT Illustrious	Crusoe
End-use group	nabim Group 1				
Scope of recommendation	UK	UK	UK	UK	UK
Fungicide-treated grain yield (% treated control)					
United Kingdom (11.2 t/ha)	101	99	98	97	97
East region (11.2 t/ha)	100	99	98	97	96
West region (11.3 t/ha)	101	99	97	98	97
North region (11.2 t/ha)	99	98	98	94	93
Untreated grain yield (% treated control)					
United Kingdom (11.2 t/ha)	86	81	76	83	74
Agronomic features					
Resistance to lodging without PGR (1-9)	7	8	8	7	7
Resistance to lodging with PGR (1-9)	8	8	8	8	8
Height without PGR (cm)	83	82	81	88	81
Ripening (days +/- JB Diego, -ve = earlier)	0	0	+1	+1	+1
Resistance to sprouting (1-9)	[5]	5	6	[6]	6
Disease resistance					
Mildew (1-9)	7	5	8	6	6
Yellow rust (1-9)	8	5	9	9	9
Brown rust (1-9)	6	8	7	6	3
Septoria nodorum (1-9)	[6]	[6]	[6]	[6]	6
Septoria tritici (1-9)	6.4	5.9	5.5	6.1	6.5
Eyespot (1-9)	7@	6@	5	6@	5
Fusarium ear blight (1-9)	6	7	6	6	6
Orange wheat blossom midge	-	R	-	-	-

United Kingdom

The UK National Lists are maintained by the Plant Variety Rights and Seeds Office (PVSO), which is part of the Animal and Plant Health Agency (APHA). Recommended or described variety lists are produced for most major crops (cereals and oilseeds, sugar beet, grass and clover, and forage maize and amenity grasses) in the UK, delivered by levy boards and industry in partnership with plant breeders through the British Society of Plant Breeders (BSPB). The Recommended Lists for cereals and oilseeds are produced by the UK levy board, Agriculture and Horticulture Development Board (AHDB), in partnership with the UK plant breeders and end-users (BSPB, Maltsters' Association of Great Britain (MAGB), and the National Association of British and Irish Flour Millers (nabim). Approximately 400 UK cereal and oilseed trials and additional quality tests are used to establish whether a new variety has a balance of features likely to give an economic benefit to the industry. The balance of features covers agronomic, quality, end use and yield traits, and many of these are presented on a scaled system (Table 3). A 1 to 9 scale is widely used to describe agronomic traits, where 9 indicates the variety shows the trait to a high degree (e.g., high resistance). This scale is used to describe the lodging and disease resistance across all recommended cereal and oilseed recommended lists, whereas due to a more limited dataset in the descriptive lists (spring linseed, spring oilseed rape, winter rye, winter triticale), lodging data are presented as percentages and disease data cannot be presented. Further agronomic traits specific to particular crops are also described on this 1 to 9 scale – resistance to sprouting (winter wheat), resistance to brackling

(spring barley), stem stiffness (winter oilseed rape), earliness of flowering (spring and winter oilseed rape, spring linseed) and shortness of stem (spring oilseed rape). The oilseed crops also present earliness of maturity on a 1 to 9 scale, whereas the cereal crops present maturity as the ripening difference in days to a control variety.

Belgium

In Belgium, the Institute for Agricultural and Fisheries Research (ILVO, Flanders) and the Walloon Agricultural Research Center (CRA-W) are responsible for carrying out variety testing. Experimental data from both regions are combined annually in a final report, which forms the basis for the registration of varieties. ILVO summarises the results for maize, fodder beets, industrial chicory, grasses and clover species and catch crops, CRA-W those for potato, wheat, barley, oats and spelt. A descriptive list of varieties is created for cereals, fodder beets, grasses and clover. This can be extended to create a recommended list for silage and grain maize. To describe some traits, scoring data (scale 1 to 9) is averaged. The score of 9 represents the maximum expression (e.g. very late maturity, very good standing ability, resistant to diseases) and the score of 1, the lowest expression (e.g. very early maturity, very low standing ability, very low disease resistance). Maturity is expressed as number of days earlier/later than a reference variety. The yield is reported in relative percentages and plant height, and the quality traits are reported in absolute values.

Bulgaria

The Executive Agency for Variety Testing, Field Inspection and Seed Control (IASAS) is responsible for carrying out the variety approval procedure. Some of the trials are run by private experimental farms. The official VCU trial takes 2 to 4 years, depending on the plant species and the testing conditions. A national catalogue (Lists A and B) is issued annually. A bulletin is issued that shows the characteristics of newly registered varieties and compares them to those of standard varieties. There is neither a descriptive nor a recommended list of varieties. A scale that ranges from 1 (very unfavourable) to 9 (very favourable) is often used during the variety testing.

Croatia

The Croatian Agency for Agriculture and Food (CAAF) is responsible for variety testing and maintaining the National Variety List. Two years are scheduled for the official trials. The recommended list of varieties is recognised by the Croatian legal system. Applicants need to file an application for their variety to appear on this list, and they cover all associated costs. This is a new system and applications have yet to be received. The post-registration trials on cereals started in the autumn of 2017. The original values are presented for the plant height and quality characteristics in the trial report on winter wheat. Otherwise, a scale that ranges from 1 (no lodging, no disease infestation) to 9 (very heavy lodging, very degree of severe disease infestation) is used for other characteristics.

Table 4: Czech Republic 2018 Recommended list for winter wheat (Selected varieties and traits, 2014-2017). Scale: 1 = very susceptible, 9 = resistant; quality group: E = elite wheat, A = quality wheat; falling number stability: + = high, 0 = medium, - = low.

Variety	Standardized mean (t.ha ⁻¹)	Bernstein	Genius	Annie	Pankratz	Fakir
Quality Group		E	E	E	A	A
Fungicide-untreated grain yield (% treated control)						
Maize region	8.57	85	90	84	93	92
Sugarbeet region Bohemia	10.50	81	81	79	86	84
Fungicide-treated grain yield (% treated control)						
Maize region	9.26	93	97	92	101	99
Sugarbeet region Bohemia	12.28	96	94	92	102	99
Zeleny sedimentation value (ml)		64	60	63	53	66
Protein content (%)		14.3	13.7	14.5	12.8	13.7
Falling number (s)		386	399	415	390	395
Falling number stability		+	0	0	0	-
Test weight (g/l)		826	807	821	810	806
Resistance to lodging (9-1)		7	6	7	8	6.5
Plant height (cm)		111	94	103	95	95
Thousand grain weight (g)		47	42	48	38	44
Powdery mildew - leaf		6	7.5	5	7	7
Leaf rust		7	5.5	6	7.5	6
Yellow rust (natural infection)		7	7	7	5	8
Yellow rust (inoculation test)		9	8	9	9	9
Year of listing		2015	2014	2014	2015	2013

Table 5: Denmark 2018 Descriptive list of varieties for winter wheat (selected varieties and traits). Scale: 1 = very low degree (very early, very short, very low tendency toward lodging, very low susceptibility to diseases etc.), 9 = very high degree (very late, very tall, very high tendency toward lodging, very strong susceptibility to diseases etc.).

Variety	tendency toward, susceptibility to							Quality traits				
	Ripening	Length of straw	Lodging	Powdery mildew	Yellow rust	Brown rust	Septoria blotch	Weight of kernel	Weight per litre of kernels	Crude protein content	Sedimentation value	Falling number
Benchmark	5	8	1	5	6	4	6	7	5	4	4	6
Creator	5	7	1	4	6	5	3	7	3	5	6	7
Hereford	5	5	2	6	2	7	7	6	5	4	3	5
Jensen	5	6	1	3	3	5	6	6	7	1	5	7
Kalmar	4	5	2	6	2	2	4	3	5	3	4	3
KWS Blanche	4	1	1	7	2	4	7	3	6	6	7	4

Czech Republic

The Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ) is responsible for variety testing and provides recommended lists of varieties of winter wheat, spring wheat, winter barley, spring barley, winter triticale, oat, field pea, soybean, winter oilseed rape, spring rape, flax, sugar beet, and potato. This system of Recommended Lists has existed since 2003, and only Descriptive Lists were available from 1994 to 2003. The results from the last two to four years are included. Because two-thirds of the results are obtained from the official VCU trials, EU varieties are currently excluded from these lists. In 2018, 122 winter wheat varieties appeared in the national list, of which 33 varieties were recommended. Independent recommendations are made for winter wheat, spring wheat, and spring barley that are cultivated on organic farms. A descriptive list of varieties is available for winter rye, spring triticale, and naked oats as well as for lupine, poppy, mustard, and cumin. However, brochures on cereals (ÚKZÚZ 2018), grain legumes, and oil plants mostly contain all of these species. To describe the varieties, a scale that ranges from 1-9 is used. A high number indicates a strong expression of the characteristic (high degree of resistance to lodging and diseases), and a low number, a low expression (low degree of resistance to lodging and diseases). The yield performance of the particular variety is compared to that of a control variety. Plant height and many quality characteristics are indicated in the form of average values (Table 4).

Denmark

In Denmark, the TystofteFoundation oversees variety testing and variety approval. This foundation is private and was founded in 2015. VCU testing is performed over two years except for forage grasses, which are tested over a three year period. Two descriptive lists of varieties (agricultural plants, amenity grasses) are published. In the first section of the agricultural list, yield performances are reported relative to checks. For most species, checks are composed of variety mixtures with three to four components. Results are reported for all trial years of presently listed varieties. For winter wheat and spring barley, results from the past 13 years are included. The yield values from the last 11 years are listed for winter barley, rye and oats and from the last 9 years for maize and winter oilseed rape. Agronomic traits and quality characteristics of the varieties are scored using a scale that ranges from 1 to 9, with 1 representing a very low expression and 9, a very pronounced expression of the studied character (Table 5). In the second section, results are given in absolute values for newly tested varieties. Disease tolerance is given by degree of disease infestation measured by percentage of infested leaf area (TystofteFonden 2018).

Table 6: France 2018 Descriptive list of varieties for winter wheat (selected varieties and traits). Scale: 1 = very late, very short, very low resistance to lodging and diseases, very low hectolitre weight or protein content; 9 = very early, very tall, resistant to lodging and diseases, very high hectolitre weight or protein content; Alveograph values: W = dough energy (area under curve), P = dough strength (maximum resistance/pressure), L = dough extensibility; quality class: BPS, superior or bread-making quality; BP, bread-making quality.

Variety	Resistance to									Quality traits				
	Early heading	Plant height	Lodging	Powdery mildew	Yellow rust	Septoria tritici blotch	Brown rust	Fusarium head blight	DON accumulation	Test weight	Protein content	W-value (11.5% protein content)	P/L-value (11.5% protein content)	Quality class
Arezzo	7	3.5	6	6	7	6	3	5.5	4.5	8	6	180-235	0.9-2.0	BPS
Boregar	6	3	5.5	6	5	6	2	(3)	4	5	5	165-175	0.6-1.4	BPS
Cellule	6.5	3.5	7.5	6	6	6.5	3	5	4.5	8	4	185-225	1.4-3.0	BPS
Fructidor	6	3.5	6.5	7	7	6.5	7	5	5.5	7	4	175-200	0.9-1.4	BPS
Rubisko	6.5	3.5	6.5	6	7	5.5	8	5.5	5	5	5	135-195	0.3-0.7	BP
Terroir	5.5	3	7.5	7	8	5	7	4	4.5	5	4	165-200	0.4-1.5	BPS

Estonia

The Estonian Agricultural Board is responsible for maintaining the official plant variety register. The VCU trials last 2 years and are organised by the ARC Viljandi Variety Testing Center as are the post-registration trials. A Recommended List that had been established in 2004 was discontinued in 2014 due to lack of success, in part due to seed availability of the most valuable varieties. Trial reports are published, and the plant height, yield and quality characteristics are presented in absolute figures. A scale that ranges from 1 (no lodging, no disease symptoms) to 9 (complete lodging, very strong disease symptoms) is in use. To describe winter hardiness, the scale is reversed (1 = poor winter hardiness, 9 = very good winter hardiness).

Finland

The Plant Variety Boards is responsible for the official approval of a variety on The Finnish Food Authority's (Ruokavirasto) recommendation. The trials are conducted by Natural Resources Institute Finland (Luke) in collaboration with plant breeding companies and other institutions. After a trial period of two years (for annual plants) or three years (for perennial fodder plants), a variety can be included in the official catalogue of varieties. There is no descriptive or recommended variety list. The website of the Natural Resources Institute provides information on the results of the VCU trials and supplementary tests. Plant height, yield and quality are reported as values, and winter damages, lodging and disease infestation are reported as percentages. There is no conversion into states of expression. The results are analysed with linear mixed models, which use all available data in the same analysis and so all varieties can be directly compared with each other.

France

Plant varieties are registered by the French Ministry of Agriculture which base its decisions on the expertise of the CTPS (The Permanent Technical Committee for Plant Breeding). The VCU trials take two years for most species, are coordinated by GEVES (Variety and Seed Study and Control Group), and are conducted partly by GE-

VES, INRA (French National Institute for Agricultural Research), technical institutes (Arvalis, Terres Inovia, ITB), and plant breeders. Following their registration, the 'Arvalis - Institut du vegetal' examines varieties of cereals, maize, sorghum, potatoes, fodder plants, flax, and tobacco and assesses their regional performance and suitability for different production systems. The Institute Terres Inovia is responsible for assessing oilseeds, grain legumes and industrial hemp, and the Technique de la Betterave (ITB) for assessing sugar beets. These institutes publish descriptive or recommended lists of varieties and the results of applied research. The wheat varieties are often classified using a scale of 1 to 9. The rate of 1 usually indicates the unfavourable expression of a trait (e.g. very poor winter hardiness, very

low resistance to lodging or diseases, very low hectolitre weight, very low protein content) and the rate of 9 indicates the favourable expression of a trait (e.g. very good winter hardiness, very high resistance to lodging and diseases, very high hectolitre weight, very high protein content). Early heading, early maturity and high growth are also given high scores, while late maturity and short growth are given low scores. Some quality properties (alveogram W-value and P/L-value) are presented in the form of absolute values (Table 6), and the grain yield is presented in the form of a relative percent (Arvalis 2018). The susceptibility of maize to stalk breakage and fusarium ear rot is expressed as a percentage. A scale that ranges from 1 (unfavourable) to 9 (favourable) is used to assess juvenile development and susceptibility to *Helminthosporium turcicum*. The winter oilseed rape recommended variety list uses a four-step scale (ranging from very insensitive to sensitive) to assess lodging and most diseases. Plant height, maturity, oil content and glucosinolate content of the varieties are verbally characterised. The disease susceptibility of sunflower (*Phomopsis*, *Verticillium*, *Sclerotinia*) is described using a six-step scale (ranging from resistant to sensitive), and the oil content is assessed using a verbal scale (ranging from low to very high). Lodging and *Sclerotinia* susceptibility of soybean varieties are assessed as in rapeseed. To characterise plant height (short to high) and protein content (low to very high), verbal scales are used. A scale that ranges from 1 (late, low, sensitive, unfavourable) to 9 (early, high resistant, favourable) is in use to assess many characteristics of potato. There are only two gradations (sensitive, resistant) used to assess susceptibility to some viruses. The tuber yield is expressed relative to that of a standard variety.

Germany

The official variety testing system used in Germany is bipartite. While the Federal Plant Variety Office is responsible for variety testing for the purposes of variety approval, Federal States are responsible for variety testing for regional variety recommendation. Depending on the species, the VCU trials take 2 to 3 years. Based on the Seed Act of 2004 (*Saatgutverkehrsgesetz* 2004, § 56), the Federal Plant Variety Office publishes several Descriptive Lists

Table 7: Germany 2018 Descriptive list of varieties for winter wheat (selected varieties and traits). Scale: 1 = very low degree (very early, very short, very low winter damages and lodging, very low susceptibility to diseases etc.), 9 = very high degree (very late, very tall, very high winter damages and lodging, very strong susceptibility to diseases etc.); falling number stability: ++ = very good, + = good, o = medium, - = poor, - - = very poor; baking quality group: E = elite wheat, A = Quality wheat, B = Bread wheat, C = Feed wheat.

Variety	tendency towards, susceptibility to							Yield characteristics			Quality traits							
	Maturity	Plant height	Winter damage	Lodging	Powdery mildew	Yellow rust	Leaf rust	Fusarium head blight	Thousand grain weight	Grain yield, intensity level 1	Grain yield, intensity level 2	Falling number	Falling number stability	Crude protein content	Water absorption	Flour yield T550	Baking volume	Baking quality group
Akteur	5	6	5	5	8	9	5	4	5	1	3	8	+	8	4	7	8	E
Apostel	5	4	-	5	1	2	4	4	7	8	6	7	o	4	3	7	7	A
Benchmark	5	4	6	4	2	4	7	5	4	7	8	7	o	2	3	7	4	B
Elixer	6	5	4	6	4	3	4	4	4	8	7	6	o	3	1	5	4	C
Julius	6	5	3	4	4	4	4	5	6	6	6	8	+	4	8	8	6	A
RGT Reform	6	3	4	4	3	4	3	4	6	7	7	9	+	4	3	7	6	A

of Varieties: cereals, maize, oil and fiber plants, legumes, beets, catch crops (Bundessortenamt 2018); potato; forage grasses, sainfoin, clover, alfalfa; amenity grasses; vines; and other species in irregular intervals.

By incorporating the results collected by the federal states (chambers of agriculture, regional institutions, etc.), variety descriptions are also available for varieties that are not registered in Germany. The properties are characterised using a scale that ranges from 1 to 9 (Table 7). Until the beginning of the 1970s (Bundessortenamt 1972), the rating of 1 was the best rating, and the rating of 9 was the worst rating for a feature characteristic. Since 1973, a low rating is given for a low expression of a feature characteristic, and a high rating for a strong expression, while a middle rating is given the rating of 5 (Bundessortenamt 1974). The phrase ‘tendency toward’ is used with reference to some agronomic traits, and the phrase ‘susceptibility to’ is used with reference to diseases and pests. The falling number stability of wheat is described in five grades (++ to - -). In the case of cereals, the grain yield and – in the case of sugar beet – the sugar yield and the sugar content are described using two intensity levels. Winter barley, spring barley, winter wheat, and oats are classified separately for organic farming.

Recommended varieties and rating scales of German federal states

To be able to select suitable varieties, the results of VCU trials, federal state variety trials, and the experience of local crop experts are essential. Classifications that deviate from the norm are published in the national variety list issued by the Federal Plant Variety Office in most federal states. This is based on a stronger weighting of the results collected in the region. In the federal states of Schleswig-Holstein, Lower Saxony, Rhineland-Palatinate, Mecklenburg-Vorpommern, Brandenburg, Saxony-Anhalt, Thuringia, Saxony, and Bavaria, signatures (+, 0, -) are used for some features, often with fewer gradations, instead of the scale 1 to 9. In Baden-Württemberg, the characteristic values are reproduced verbatim, but in abbreviated forms. In North Rhine-Westphalia

and Hesse, some agronomic features and diseases are combined with the phrases ‘tendency toward’ or ‘susceptibility to’. Otherwise, the opposing phrase ‘resistance to’ are mostly in use. The yields are always reported by relative percentage. In 2018, the Bavarian State Research Center for Agriculture described 32 out of the more than 150 winter wheat varieties cited in the National List as suitable for conventional cultivation, using signatures that ranged from +++ to - - - (Table 8) and recommended 10 to 12 varieties (Hartl & Nickl 2018). The descriptive list for organic farming in Bavaria included 30 winter wheat varieties, of which 13 were recommended. In the organic list, plant height is described as +++ = very tall and - - - = very short, which is different to conventional production conditions where the opposite scale is used.

Hungary

The National Food Chain Safety Office (NÉBIH) tests and approves plant varieties, but there is no descriptive list of varieties. The VCU trial results are published in the form of reports. Agronomic data, grain yields and quality results are mainly presented as absolute values. Properties such as hibernation, lodging and disease infestation are scored on a scale that ranges from 1 (unfavourable) to 9 (favourable) in use. For several years, the Grain Producer's Association, the National Seed Association and the Chamber of Agriculture have organised post-registration trials for wheat and grain maize. In the report on maize, grain yields are also described and scored with scores ranging from 1 (very low yield) to 9 (very high yield). In 2018, the Chamber of Agriculture proposed a Recommended Variety List for maize, and they plan to issue such a list for wheat in 2019.

Ireland

The Department for Agriculture, Food and the Marine (DAFM) organise variety trials in Ireland. Varieties are evaluated and recommended variety lists are created with reference to the results. The system has existed in this form since the 1970s. There are recommended lists for winter cereals, spring cereals, spring

Table 8: Bavaria 2018 Descriptive list of varieties for winter wheat for conventional production conditions (selected varieties and traits). Scale: +++ = very good, very high, very early, very short; o = medium; --- = very poor, very late, very tall; falling number and falling number stability rates from the German Descriptive List 2018; falling number stability: ++ = very good, + = good, o = medium, - = poor, -- = very poor.

Variety	Baking quality group	Resistance to							Quality traits					
		Maturity	Plant height	Standing ability	Powdery mildew ¹⁾	Septoria tritici blotch	Yellow rust	Leaf rust	Fusarium head blight	Thousand grain weight	Falling number ¹⁾	Falling number stability	Crude protein content	Crude protein content (%)
Expo	E	o	(-)	o	+	(+)	+	(-)	+	(+)	++	+	(+)	13.3
KWS Emerick	E	o	o	(+)	+	(+)	++	o	(+)	+	++	+	(+)	13.4
Beryll	E	o	o	+	++	(+)	+	++	o	o	+	o	++	14.2
RGT Reform	A	(-)	+	(+)	+	o	(+)	(+)	(+)	(+)	+++	+	o	12.8
Nordkap	A	o	o	(+)	+++	o	+	(-)	o	(+)	+	o	(+)	13.2
Julius	A	(-)	o	(+)	(+)	(+)	(+)	-	o	(+)	++	+	o	13.0

beans, winter oilseed rape, forage maize, ryegrasses and white clover. At least three years of testing are necessary for a variety to be 'provisionally recommended'. In general, they are given the status of 'recommended' after the fourth year of testing. In the case of potato, there is no Recommended List, and the tests are carried out with regard to the variety listing of the National Catalogue of Agricultural Plant Varieties. The yield assessments are presented in values that are relative to the average of the values of the control varieties. Absolute values are provided for the plant height and quality parameters such as thousand grain weight, hectolitre weight, screenings, protein content and Hagberg falling number. A scale of 1 to 9 is used when assessing subjective features such as earliness of ripening, stem stiffness, resistance to lodging or resistance to diseases, with 1 being the least favourable and 9 the most favourable score (Table 9, DAFM 2018).

Latvia

The Latvia University of Life Sciences and Technologies (LLU) has been commissioned to carry out variety testing. The results are available in the form of a report. The yield performance values are reported in absolute terms and in relative percentages. The total yield calculated on standard moisture grain 14%, rape seed 8%, then the yield compared with the standard variety or average yield of standards,

Lithuania

The State Plant Service under the Ministry of Agriculture (VATZUM) is responsible for variety testing and variety registration. Recommended and descriptive lists are not produced, however, descriptions of the characteristics of the new varieties appear on the published National List of Varieties. Trial reports contain the results for assessments of many features (e.g., plant height, yield, quality characteristics, and resistance to diseases). Furthermore, a nine-step scale is in use where 9 indicates the favourable expres-

sion depending on crop in percentages and according % given rates. For characteristics such as winter hardiness and lodging resistance, the scores are calculated as ranging from 1 (very low, very poor) to 9 (very high, very good). The averaged absolute values for the quality parameters are given scores that range from 1 (very low) to 9 (very high) according to the tabular specifications. The disease infestation of potato is given as a percentage.

Table 9: Ireland 2019 winter wheat recommended list (selected varieties and traits). Scale: 1 = least favourable, 9 = most favourable. Yields are expressed as a percentage of the mean JB Diego and Avatar (100 = 11.21 t ha⁻¹ at 15% moisture content). All data based on trial results from 2016-2018 with the exception of falling number (2016-2017). End-use quality: Br = bread-making potential, F = feed quality.

Agronomic & quality characteristics	Avatar	Bennington	Costello	KWS Lili	KWS Lumos
Relative yield	100	103	100	99	99
Straw height (cm)	78.9	79.1	69.3	69.0	71.5
Resistance to lodging	7	7	8	8	8
Earliness of ripening	5	6	6	4	7
Resistance to:					
Powdery mildew	5	6	8	8	7
Yellow rust	7	6	8	6	7
Fusarium ear blight	6	7	7	4	5
Sprouting	4	5	8	6	7
Quality:					
Grain protein (% 15% MC)	10.2	10.7	10.8	10.3	10.8
Hagberg falling number (s)	188	185	290	283	287
Thousand grain weight (g)	46.9	48.5	46.7	43.9	43.8
Test weight (kg/hl)	77.1	76.4	79.4	76.2	77.8
Market	F	F	F	Br	Br
Year first listed	2013	2018	2017	2016	2014

Table 10: The Netherlands 2018 Recommended list for winter wheat (selected varieties and traits). Scale: 2 = very unfavourable (very low lodging resistance, very late ripening, very low resistance to sprouting and diseases, very low baking quality etc.), 9 = very favourable (very high lodging resistance, very early ripening, very high resistance to sprouting and diseases, very low bread quality etc.); A = recommended for general or fairly general use; N = new, provisionally recommended; B = bread wheat

Variety	Relative straw length (100=91.9 cm)	Strength of straw	Earliness of heading	Earliness of ripening	Resistance to						Bread-making quality				
					Sprouting	Yellow rust	Leaf rust	Powdery mildew	Leaf spot (<i>Septoria tritici</i>)	Fusarium in the ear	Bread quality	Dough quality	Zeleny sedimentation value	Hardness of kernel	Classification
A - Julius	108	8.0	5.5	5.5	7.0	7.0	6.5	7.0	8.0	6.5	8.0	7.0	8.0	8.0	B
A - Kelvin	101	7.5	7.0	6.5	7.5	8.0	9.0	7.5	7.5	6.5	7.5	6.5	7.0	7.0	B
A - RGT Reform	95	8.0	6.5	7.0	7.0	7.0	8.0	7.5	7.0	7.0	8.0	9.5	8.0	7.0	B
N - KWS Talent	111	7.0	7.5	7.0	7.0	8.5	8.0	7.5	7.5	7.0	7.0	7.0	7.0	7.5	B
N - RGT Libravo	94	7.5	7.0	7.5	7.5	8.5	6.5	7.0	6.5	7.0	7.0	7.5	7.0	6.5	B
N - WPB Calgary	98	7.0	8.0	7.0	6.5	8.5	8.0	8.5	7.5	6.5	7.0	7.0	7.0	7.0	B

sion of a trait (high degree of winter hardiness, very good standing ability, high degree of disease resistance) and 1, the unfavourable expression of a trait (very low degree of winter hardiness, very poor standing ability, very low degree of disease resistance).

Luxembourg

Variety trials are carried out in Luxembourg by the Lycée Technique Agricole (LTA), the Administration des services techniques de l'agriculture (ASTA) and the Institute for Organic Agriculture Luxembourg (IBLA). The results are used for variety approval and form the basis of the descriptive list of varieties. A scale that ranges from 1 to 9 is in use, whereas the score of 1 indicates a very low expression of a trait (very low, very early, very short) and the score of 9 indicates a very strong expression (very high, very late, very tall). In the case of cereal diseases 'resistance' (1 = very low resistance, 9 = very high resistance) is described; regarding potato diseases, however, 'susceptibility' is described (1 = very low susceptibility, 9 = very strong susceptibility).

The Netherlands

In the Netherlands, the Board for Plant Varieties can approve a variety after two years of VCU trials and add it to the National List. After a third year of testing, the Recommended List Committee (CSAR) decides whether the variety is eligible for placement on the recommended list. The two recommended lists (arable crops, forage crops) describe varieties, scoring them with a scale that ranges from 2 to 9 (CSAR 2018). In general, the score of 9 represents a very favourable expression of the trait (e.g., very good stem strength, very high resistance to sprouting and diseases) and a score of 2, a very unfavourable expression (e.g., very low stem strength, very low resistance to sprouting and diseases). Early ear emergence and early ripening are also expressed by high scores. Plant height and yield are usually given in the form of relative

values. The quality data are partly relative and partly absolute (Table 10).

Norway

The Norwegian Food Safety Authority (Mattilsynet) is responsible for the variety approval and the publication of the National Variety List. The VCU trials last three years and are performed by the Norwegian Institute of Bioeconomic Research (NIBIO) on behalf of the Norwegian Food Safety Authority. A descriptive or recommended list of varieties in the true sense does not exist. However, the results of official VCU trials and post-registration tests are reported in detail. The publications summarise the data that has been collected during the past year and the last three years (Åssveen et al. 2018). The grain yields are reported as relative percentages, plant height and quality parameters are reported in absolute values. Characteristics such as lodging, brackling and diseases are given as percentages. In addition, the results are also converted to fall along a scale of 1-10. The score of 1 represents high growth, low standing ability, a low degree of disease resistance, low thousand grain weight and low quality. The score of 10 is awarded to plants with short growth, good standing ability, a high degree of disease resistance and high quality.

Poland

The Research Center for Cultivar Testing (COBORU) is responsible for testing and approving plant varieties. Depending on the species, the VCU tests take 2 to 3 years. Descriptive lists of agricultural plant varieties, vegetables and fruits are published. The Descriptive List of Agricultural Plant Species contains results from the last 3 to 4 years (COBORU 2018). New varieties are also verbally characterised. Disease susceptibility is scored using a scale that ranges from 1 (very high infestation) to 9 (no infestation), and averaged scoring data is used. Quality features are occasionally represented by absolute values, but the data is predominantly transformed

Table 11: Poland 2018 Descriptive list of varieties for winter wheat (selected varieties and traits). Scale: 1 = very unfavourable (very low lodging resistance, very low resistance to diseases, very low quality), 9 = very favourable (very high lodging resistance, very high resistance to diseases, very high quality)

Variety	Plant height (cm)	Lodging resistance	Fully ripe (d from Jan 1 st)	Resistance to				Quality traits								
				Powdery mildew	Leaf rust	Yellow rust	Fusarium head blight	Thousand grain weight (g)	Test weight	Protein content	Gluten Index	Flour yield	SDS sedimentation value	Water absorption of dough	Bread volume	Dough energy
Elite wheat (group E)																
Astoria	98	7.4	203	7.3	7.6	8.1	7.5	49.5	6	8	7	5	8	9	8	9
Quality wheat (group A)																
Apostel	89	7.0	204	8.1	7.8	8.7	7.3	47.2	6	5	5	5	8	8	7	-
Arkadia	102	6.7	203	6.4	7.7	5.8	7.6	47.7	5	5	7	5	7	9	7	7
Arktis	94	7.7	204	7.9	7.1	8.7	8.0	44.4	5	7	8	6	8	9	7	9
Askalon	87	8.5	205	7.7	7.9	8.7	8.1	43.2	5	5	8	5	8	8	6	7
Bamberka	91	7.7	205	7.9	7.2	8.6	7.6	46.9	6	7	8	5	8	9	6	8

into scores that range from 1 (very unfavourable) to 9 (very favourable). Plant height is usually provided in centimeters and the maturity, in days (Table 11). Since 1998, there have been post-registration trials with newly approved and important varieties on the market. EU varieties can only be included in this test if they have previously been included in the 'EU recognition trials' for two years. In 2018, this system included 973 field trials that involved more than 750 varieties. To receive a recommendation, two years of results from post-registration trials are generally needed. If excellent results are available, a recommendation can already be made after a total of three years of testing. Based on this approach, local committees (since 2004), prepare lists of recommended varieties for cultivation in the voivodeships. In 2018, there were regional variety recommendations for winter cereals, spring cereals, grain maize, silage maize, pea, lupines, soybean, winter oilseed rape, spring oilseed rape and potato.

Romania

The State Institute for Variety Testing and Registration (ISTIS) is responsible for the variety approval and creating the list of varieties. The official trials usually take three years. Newly registered varieties are described in a separate chapter of the official catalogue of varieties. It is legally possible to create a recommended list of varieties; while some attempts to do so have been made, no such list has been published so far. The expression of some features are assessed using a nine-step scale. In this case, the score of 1 refers to the favourable expression (e.g., no overwintering, no lodging, no disease infestation) and 9, the unfavourable expression (e.g., very strong overwintering, very heavy lodging, very severe disease susceptibility). To assess disease susceptibility, a percentage scale is sometimes used. To assess the turf grasses, the scale is reversed (1 = very weak, 9 = very favourable).

Serbia

The registration of plant varieties is carried out by the Ministry of Agriculture, Forestry and Water Management. The Department for Plant Variety Registration and agricultural professional services carry out the required VCU trials. There are no descriptive or recommended variety lists. Agronomic data, yield and quality results are published in trial reports. A scale that ranges from 1 (favourable, e.g., no winter damage, no lodging, no disease infestation) to 9 (unfavourable, e.g., very heavy winter damage, very heavy lodging, very severe disease infestation) is used to describe some cereal traits. The scale is reversed when describing traits of legumes and forage crops, with the score of 1 representing the unfavourable expression (very heavy winter damage, very heavy lodging, very severe disease infestation) and the score of 9, the favourable expression (no winter damage, no lodging, no disease infestation).

Slovakia

In Slovakia, the Central Controlling and Testing Institute of Agriculture (ÚKSÚP) is responsible for organizing and carrying out VCU trials and registering varieties. Post-registration trials are not performed. Varieties are rated on a scale that ranges from 1 (highly susceptible) to 9 (resistant) regarding disease classification. For other variety characteristics, the averages of the absolute values are given, and these are partly supplemented with rates (1 = unfavourable, 9 = favourable). A descriptive or recommended variety list is not currently published.

Slovenia

The Ministry of Agriculture, Forestry and Food approves varieties and publishes the national variety list. The official VCU trials and other variety trials are carried out by the Agricultural Institute of Slovenia (KIS). There are descriptive lists for varieties of cereals (winter wheat, winter barley, rye) as well as for maize and potato.

These also include EU varieties, but the lists are not issued annually. The systems used for description and scaling are not unified. Cereals are often characterised using a verbal scale (low to high, poor to good). Trial reports include data on plant height, grain yield and quality of cereals in absolute values. Otherwise, a grading scale that ranges from 1 (favourable, meaning no lodging, no disease infestation, etc.) to 9 (unfavourable, meaning very heavy lodging, very severe degree of disease infestation, etc.) applies. In maize, the properties are mostly assessed using signatures that range from - (e.g., low growth, low resistance to stem breakage and diseases, low grain yield, high grain moisture at harvest) to +++ (high growth, high resistance to stem breakage and diseases, high grain yield, low moisture at harvest). Some properties of potato varieties are communicated using a verbal scheme. To describe disease susceptibility, a scale that ranges from 1 (very high susceptibility) to 9 (very low susceptibility) is used. The international EAPR rating scheme is used to describe quality characteristics.

Spain

The National Register of Varieties is produced by the Spanish Plant Variety Office (OEVV). The public organisation Grupo para la Evaluación de Nuevas Variedades de Cultivos Extensivos en España (GENVCE) carries out trials for cereals, oilseed rape and maize and publishes reports on variety performance across agroclimatic regions. Results from one or two years of data are often summarised. Plant height, date of heading, grain yield and quality are reported in absolute or relative values; lodging as well as disease infestation

are reported as percentages. A scale that ranges from 0 (no infestation) to 9 (very strong infestation) is also in use to assess diseases and pests of cereals. Furthermore, summary tables in which the varieties are described on a verbal scale (low to high, early to late) are used for some plant species.

Sweden

The Swedish Board of Agriculture is responsible for the registration of plant varieties, and the Swedish University of Agricultural Sciences (SLU) is responsible for VCU testing of these varieties. Admission requires at least two years of trial results (grasses are tested with two establishment year with following harvesting years). Trial reports on cereals, maize, grain legumes, oil plants, flax and potatoes are published yearly. Since these mostly include results from the past five years (except forage species which have past 10 years), the reports are somewhat like a descriptive list of varieties, but are not referred to as such. Forage grasses and legumes are also tested. Yield performance and quality characteristics are described with absolute values, whereas overwintering is presented on a percentage scale that ranges from 100 (plants all present) to 0 (no living plants present). The same applies to lodging and stem breakage (0 = no lodging or stem breakage, 100 = all stalks display lodging or are broken). With regard to diseases, the percentage of affected leaf area is cited (Halling & Larsson 2017, Hagman et al. 2018). Considerations are being made to use optional scores that range from 1 to 9 to describe some characteristics.

Table 12: Swiss list of recommended winter wheat varieties for the harvest 2019 (selected varieties and traits). Scale: +++ = very good; \emptyset = medium; --- = very poor; Extenso: low input (without fungicides, insecticide and growth regulators); ÖLN: medium input (with fungicides - if needed - and growth regulator, without insecticide); Ripening: ve = very early; e = early; me = mid-early; l = late; Plant height: s = short; m = medium; ml = medium to tall; vl = very tall

Variety	Arnold	Montalbano	Baretta	Forel	Simano
Quality class	Top	Top	Top	I	I
Year of listing	2015	2018 (prov.)	2018 (prov.)	2008	2012
Grain yield (Extenso)	\emptyset	+	+(+)	\emptyset	+
Grain yield (ÖLN)	\emptyset	+	+	+	+
Early ripening / Heading	e	l	l	me	ve
Plant height	vl	m	ml	m	s
Resistance to:					
Lodging	-	+++	++(+)	++	+++
Powdery mildew	+(+)	++	+(+)	+(+)	+
Yellow rust	\emptyset	+++	+++	\emptyset	+
Leaf rust	\emptyset	++	+(+)	--(-)	+
Fusarium head blight	\emptyset	\emptyset	--	-	+
Sprouting	\emptyset	+	-	+	+
Quality traits:					
Protein content	++(+)	+++	++(+)	+(+)	++(+)
Zeleny sedimentation value	++	++	++	++	++
Test weight	+++	+	\emptyset	+++	+(+)
Thousand grain weight	medium	large	small	very small	medium

Switzerland

The Federal Office for Agriculture registers varieties and includes them in the National Catalogue of Varieties. The two-year VCU trials are carried out by Agroscope. The post-registration trials also require two years and are organised by Agroscope in collaboration with members of the agricultural sector. There are various lists of varieties that are created by companies and trade organisations in Switzerland. For conventional production and organic farming purposes, official lists of recommended varieties are created or supported by Agroscope. These are developed in cooperation with the organisations swiss granum and swisspatat. These lists are considered to be particularly valuable and they are trusted references for the production of various labels. Swiss granum lists of varieties have existed, for example, since 2000. Varieties for the following species have been described and recommended: cereals (Courvoisier et al. 2018), maize, soybean, winter oilseed rape, sunflower, lentils, potato and forage crops. The presentation (Table 12) is usually made with signatures that range from ++++ (excellent, grain yield of winter wheat) to +++ (very good) and on to --- (very weak). Potato varieties are described verbally in terms of their properties. Varieties of forage plants are predominantly listed with their averaged scoring values. A review of 15 years of variety testing has shown (Levy et al. 2017) that around one-third of the winter wheat varieties admitted for entry in the catalogue of varieties are ultimately placed on a list of recommended varieties. Of

the varieties that are not placed on the latter list, about half did not perform adequately in the VCU trials, and the other half were assessed as not viable for inclusion in the list of recommended varieties. The success rate of varieties registered abroad was even lower, with less than a quarter making it onto the list of recommended varieties. Seed propagation is also based on these official lists. Representatives of the entire sector, including the umbrella organisation of the seed propagators, decide which varieties will be recommended. The voting rights are distributed equally among producers and customers (e.g., millers, bakers).

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Genotypic selection in wheat on the field: an optimized overall concept

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Abstract

Consumers are demanding natural and high-quality bread and pastry products, and farmers want to produce winter wheat of high quality in an environmentally friendly, sustainable and economic efficient way. The breeders are required to breed varieties which combine many traits in the best possible combination. Therefore, an overall concept '*Life cycle of a wheat variety from the crossing to the deletion of the variety*' was developed. To achieve this aim, the path of the variety must be optimized in all steps, and succeeded. The three basic requirements for successfully developing a new variety, *i.e.* 'crossing', 'guiding the crossing' and 'test sites', must be optimized. The selection of the crossing partners, the selection steps in the segregating and homogeneous generations, and the selection of lines for the VCU (value for cultivation and use) test were implemented in the wheat cultivars 'Capo', 'Georg' and 'Josef' registered in 1989, 1992 and 1993, respectively. Moreover, production-specific small strip plot experiments were established by Probstdorfer Saatzucht on trial sites across Central and Eastern Europe. The aim was to eliminate by statistical analysis the impact of the environment on the phenotype as much as possible which enabled the 'genotypic' selection on the field. In the optimized overall concept, all connections and cross-links were linked to the 'way of life of a wheat variety'. These basic findings will ensure large economic benefits for breeders and farmers for 25 million hectares of winter wheat acreage in the continental production area of Central and Eastern Europe.

Keywords

Breeding · multi-environment trial · production specific management · *Triticum aestivum* · value for cultivation and use

Introduction

The contribution of sustainable breeding improvements in complex traits such as yield, end-use quality and yield stability in self-pollinating crops, especially winter wheat, is essential for global food security. Yield and quality at the farmer's level need to be achieved with the lowest possible input of pesticides, in order to guarantee food and feed safety and high natural quality properties both in organic and conventional wheat production. Therefore,

strong links are required between breeders, official variety testers, producers, processors and consumers.

At present, the cooperation between the individual supply chain partners is either interrupted or only loosely present. In practice, many new varieties fail after their registration in grain yield and/or in resistance characters at the farmers' fields because they were not checked in time for their production-specific management. The new varieties are indeed tested in the VCU (value for cultivation and use) trials, but parallel production-specific trials are missing, which means that the newly released varieties are starting to exhibit their practical cultivation value at the farmers' fields without additional information for the farmers. Furthermore, wheat varieties are not examined in detail for their blending suitability which can cause problems at the processors' level.

For an environmentally friendly, sustainable and economic efficient supply chain it is, therefore, necessary that a holistic and common path is sought. In particular, consumers are demanding a sustainable production. The individual supply chain members are doing a good job within their system, however, it is necessary to optimize the connections between the supply chain partners.

Climate change needs wheat varieties with a high ecological adaptation. In the last decade, the annual average temperature increased by nearly 2°C in Europe compared to the pre-industrial level. Especially during the important growth stages of wheat, from April to June, multiple stress situations occurred more frequently in the last decade. Austrian wheat varieties released 20 to 30 years ago had a high ecological adaptation because these varieties contained mainly 'continental genes' from the Central and Eastern European wheat gene pool. The problem nowadays is that in recent years this genetic was largely replaced by 'maritime genes' and associated inferior winter hardness from the Western and Northwestern Europe gene pool.

The aim of this contribution is to present an optimized master plan for the life cycle of a variety, from the crossing to the deletion from the variety list, via the genotypic selection on the field.

Breeding concept

In the short term, it is necessary to select genotypes adapted to the prevailing climate from the existing breeding material and to recommend the site and variety specific management to the far-

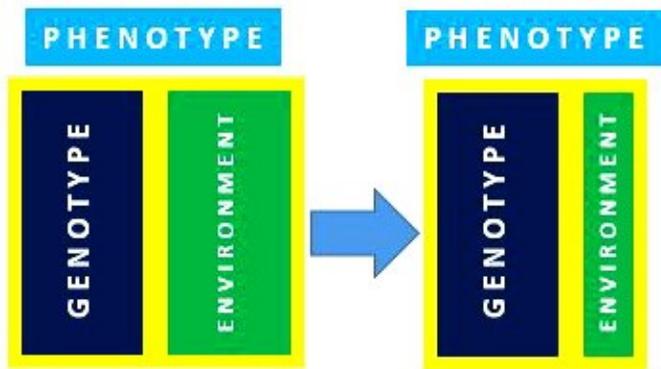


Figure 1: Concept of genotypic selection on the field: The goal is to reduce the impact of the environment on the phenotype. If all environmental impact is eliminated the genotype can be directly selected on the field.

mer. In the medium and long term, it is necessary to develop varieties broadly adapted to climate change.

The term 'genotypic selection on the field' is only permitted if all environmental impact on the genotype is eliminated, thus, not the 'phenotype' but the 'genotype' is explained (Fig. 1) (Velu & Singh 2013). The overall concept was first introduced by Grosslercher on 16th January 2016 in a talk at the 'Exchange for Agricultural Products Vienna'. Grosslercher worked as a plant breeder for over 40 years on this first optimized overall concept, which applies to varieties, to a single test site, to multiple test sites with different production conditions, to organic and conventional farming.

Experimental designs and statistical analysis

Experimental designs with a low number of check varieties or with a very low number of replications of check varieties in order to save plots should be refused. Statistical analyses aimed to achieve a low standard error of difference or high significancies should be rejected as 'inappropriate' conclusions are drawn and predominantly 'unwanted' genotypes are selected.

Crossings and parental selection

Until the 1990s, the breeding of high baking quality wheat varieties in Austria used mainly 'continental genes', e.g. 'Capo' (released 1989) was a cross within own germplasm (Martin/Pokal) in 1975, 'Georg' (released 1992) was a cross between own and foreign germplasm (Expert/Severin) in 1983, and 'Josef' (released 1993) was a cross within own germplasm (Extrem/HP35719// Pokal/3/Perlo) in 1983. 'Expert' was a high yielding half-sister of 'Capo', 'Severin' a German elite wheat variety which brought new genes to the existing gene pool.

It is essential that for the Central European production area, mainly 'continental' genes are incorporated, which allow to survive cold winters and withstand high temperatures and drought in early summer, and, thereby, enable a broad ecological adaptation. Foreign germplasm was absolutely necessary to introgress, e.g. 'maritime' genes for enhancing grain yield or dwarfing genes to reduce plant height and, thereby, improve lodging tolerance and the harvest index. The before mentioned varieties 'Capo', 'Georg', and 'Josef' are climate-adapted wheat varieties.

Selection in segregating generations

Selection in early generations was carried out at the breeding station Probstdorf. The alluvial soils north of the Danube wetlands ensured a selection for high ecological adaptation, yield and yield

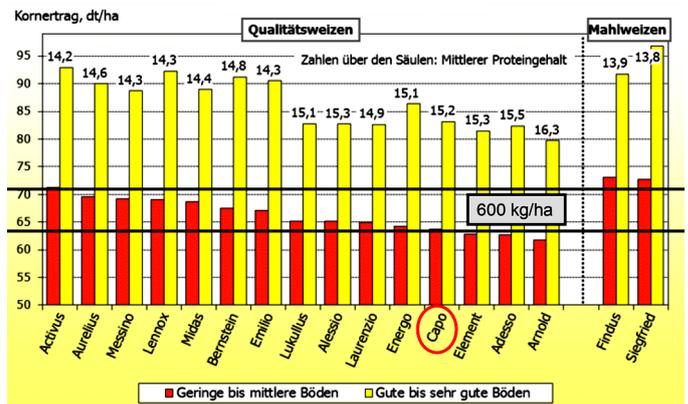


Figure 2: Grain yield of winter wheat (2013-18) in the Pannonian production area of Austria. Yellow and red bars represent yield levels at sites with high and low soil fertility, respectively. Values above bars indicate the mean protein content. Left of the dotted line, varieties of quality classes 7 to 9 are listed, to the right classes 6 and 4 (Findus and Siegfried, respectively) (Source: AGES 2018).

stability and baking quality. Grain grading and the visual assessment of grain characters is important from F₃ to F₆. Due to spatial variation in the fields, it is necessary to include check varieties regularly in several replications in the experimental designs. Thereby, differences between breeding material and check varieties can be reliably evaluated. The breeding material was selected until F₅ based on grain grading, disease resistance, plant height and the habitus of the plants.

Selection in advanced generations

In F₆, the breeding lines were sown in 1 m² micro-plots. Selection was based on grain yield and Zeleny sedimentation value. For example, the 1 m² plot of P637.88 ('Georg') and 'Josef' were harvested as bulk in 1988. Promising lines were selected only on the grain yield mean, observation data, visual kernel characteristics and the Zeleny sedimentation value without multi-environment testing. In F₇, a plot of 10 m² was sown in a replicated control design and harvested in 1989 as bulk. Parallel, single ears were selected for reselection and building up seed multiplication of pure lines. In F₈, trials were carried out with three replications at two sites and at the same time P637.88 entered the first year of VCU testing. After three VCU years all requirements for a new variety were fulfilled and, thus, 'Georg' was registered in December 1992, after only 9.5 years from crossing.

VCU and DUS tests

The VCU (value for cultivation and use) test for winter wheat is carried out for three years at several test sites across the Austrian wheat growing area. A new variety has a national value for cultivation and use if in the entirety of its traits it represents an improvement compared to the check varieties with respect to (i) the cultivation, in particular considering disease resistance, (ii) the utilization of the crop and (iii) the utilization of processed goods from the crop (AGES 2018).

'Georg' passed VCU tests successfully from 1990 to 1992, 'Josef' from 1991 to 1993. Selection in advanced generations was focused on grain yield and yield stability, gluten quantity and quality, pre-harvest sprouting resistance and on resistance against biotic and abiotic stress factors.

'Capo', 'Georg' and 'Josef' have and/or had a long lifespan at the farmers' fields because of high yield, high baking quality, good disease resistance and a high ecological adaptation. Although

already more than 25 years old, 'Capo' is still competitive compared to recently released varieties (Fig. 2). 'Capo' was originally selected as a F₃ ear progeny and a reselection was started from the 2nd VCU year onwards. Up to 10% extra grain yield can be realized with 'Capo' at productive sites in case that growth regulators are applied and plant height is reduced from 130 cm to 95 cm, thus, making this variety even more competitive still today.

Performance at farmers' fields

From the 1st VCU year onwards, the performance of 'Capo', 'Georg' and 'Josef' has already been checked on farmers' fields. Hence, even before the variety registration, their suitability for specific sites and conditions, as well as their end-use quality and processing characteristics were already well known. While 'Capo' and 'Josef' were still cultivated by farmers in 2019 in Austria and Romania, respectively, both of them after more than 25 years since their release, 'Georg' was deleted from the national list after the yellow rust epidemics in 2000 as this variety was susceptible to the prevailing races.

Premium winter wheat

With the accession of Austria to the EU in 1995, it was necessary to re-evaluate the baking quality groups of wheat. The baking quality scheme from 1994 groups wheat varieties into 9 quality classes (Oberforster *et al.* 1994). For trading, some quality groups form together specific market classes, e.g. baking quality groups 7 to 9 are traded as 'quality improver wheat'. For export, especially to Italy, the new market class 'premium wheat' which is characterized by protein contents $\geq 15\%$, falling numbers ≥ 280 s and test weights ≥ 80 kg hL⁻¹ was introduced. Additionally, the alveogram is considered as rheological test method (AGES 2018). Therefore, not all varieties of quality groups 7 to 9 fulfill the requirements for 'premium wheat'. The export success of Austrian 'premium wheat' to Italy is important for Austrian farmers. 'Capo' and other varieties of the same gene pool are today noted as 'austriaco - qualità 1^a - p.s. 79, prot. 15%' at the Exchange for Agricultural Products in Bologna.

Production

Using production-specific small plot trials at suitable test sites in Austria and Ukraine and with suitable N-fertilization levels and seeding rates, Tasheva (2016) proposed a strip plot design to select winter wheat varieties for the Central and Eastern European wheat production areas. Thereby, the seeding rate is selected trial site specific and nitrogen fertilization is applied in three levels, i.e. N1 (no fertilization), N2 (60/60/0 kg ha⁻¹ at tillering, stem elongation and heading, respectively), and N3 (60/60/60) to assess soil fertility. The characteristics of the breeding lines are checked in such trials from the first VCU test year onwards and continued until the deletion of the varieties from the national lists. Thereby, location and variety specific recommendations can be provided to farmers.

Design and analysis of small plot trials

The 'production-specific small plot trial' is designed as a strip plot trial with check varieties at regular distances, e.g. three checks regularly after 10 test lines (Fig. 3). Statistical analyses are performed according to the strip plot design. Thereby, the data can be interpreted meaningfully and location and variety specific management recommendations can be better explained to the farmers for different site conditions.

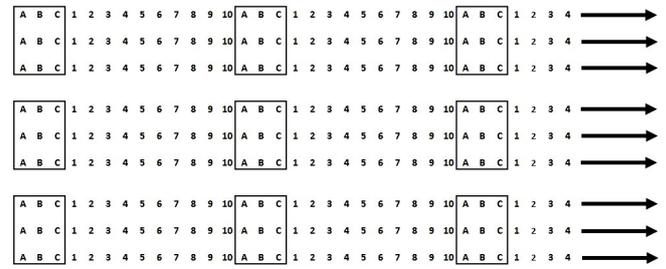


Figure 3: Production-specific strip plot trial with three check varieties (A,B,C), three replications and three production intensities, i.e. nitrogen levels.

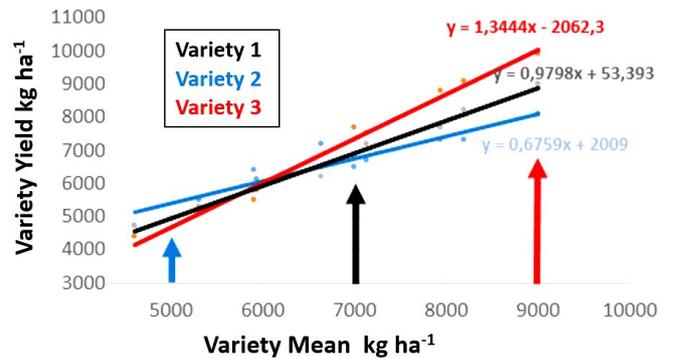


Figure 4: Linear regression of variety yield on the means of three winter wheat varieties in ten fertilization trials without late nitrogen fertilization.

Grain yields can vary from 4000 to 8000 kg ha⁻¹ for different test sites, but also within a trial area, for one and the same check variety. Hence, it is necessary to carry out partial analyses, since the yield reactions of varieties and lines are different, depending on the yield level. A trial site network of 10 to 15 trials across Central and Eastern Europe is proposed.

The varietal reaction across trial sites can be calculated by linear regression of the variety yield on the mean of three winter wheat varieties in various production trials. Varieties with a slope $b < 1$ are described as 'extensive' varieties, varieties with $b > 1$ as 'intensive' varieties (Fig. 4) (Haufe & Geidel 1978).

If the breeder selects for grain yield at low production level, e.g. due to extreme drought, according to the regression more 'intensive' lines, i.e. variety 3 in Fig. 4, are eliminated, as in this case the 'extensive' variety 2 is more productive. If the breeder selects for grain yield at high production level, more 'extensive' lines such as variety 2 in Fig. 4 are eliminated. Such an analysis can be carried out, if suitable, also as exponential regression analysis.

Of special interest for the wheat breeder is the negative correlation between protein content and grain yield (Simmonds 1995, Oury & Godin 2007, Oberforster & Werteker 2011). If the breeder selects high protein lines, 'intensive' lines are eliminated relatively independently of the trial yield level, because in this case the 'extensive' variety 1 is about 1-2% higher in protein than 'intensive' variety 2 (Fig. 5).

With respect to harvest index, field trials in Austria and Ukraine revealed that the trial sites vary according to N fertilizer management (Fig. 6) (Tasheva 2016). In Leopoldsdorf, soil fertility has been sustainably improved over decades, which means that high levels of N are also available if no N is applied (N1), which improved the harvest index to ≥ 0.4 . Although there was excellent black

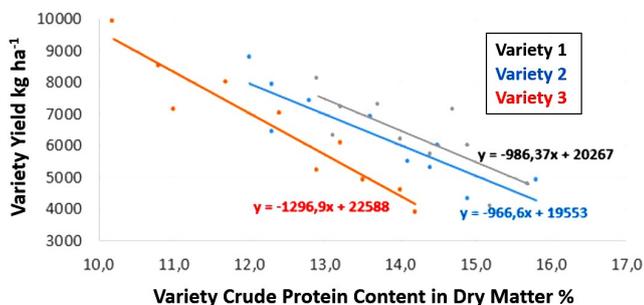


Figure 5: Linear regression of crude protein content on grain yield of three winter wheat varieties in ten trials without late nitrogen fertilization.

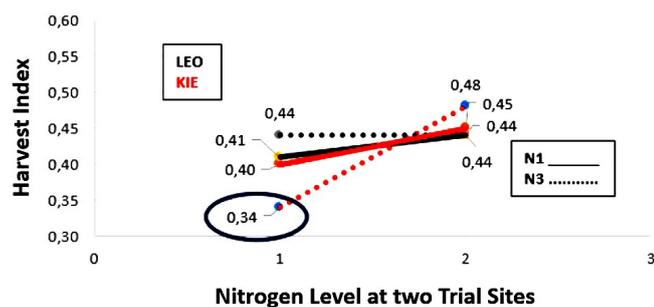


Figure 6: Harvest index for six winter wheat varieties for two fertilization levels (N1, N3) at Austrian test site Leopoldsdorf (LEO) and Ukrainian site Kiev (KIE) (trial years 2012 and 2013).

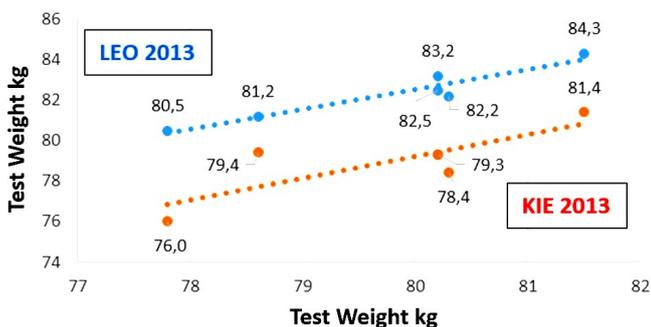


Figure 7: Linear regression of test weight (kg hL⁻¹) of two single trials in 2013 on the test weight mean from nine trials over four N fertilization regimes for six winter wheat varieties.

soil in Kiev, the availability of nitrogen was not sustainable without N fertilization, resulting in a harvest index of only slightly >0.3. This differences can be used to select lines/varieties that respond differently to N levels. Thereby, lines/varieties can be selected which achieve acceptable yield and protein content at even below-average N supply. The harvest index can be used to estimate soil fertility and/or N remobilization in the grain filling period and provides an assessment of winter wheat lines and varieties for organic and conventional farming under Central and Eastern European conditions.

For test weight, wheat varieties basically react similar, although there are large annual differences in test weight at the same test site (Tasheva 2016). In Fig. 7 it is shown that at Leopoldsdorf, which normally produces very high test weights, varieties have different levels. In the case of drought, variety means can be signi-

ficantly lower compared to Kiev, where, however, test weights are usually lower.

Conclusion

In recent decades, many medium-sized winter wheat breeders ended their breeding activities because they no longer had the necessary breeding success. The termination of breeding activities is due to different causes, e.g. unsuitable trial sites for selection in segregating and advanced generations or unsuitable experimental designs, inappropriate experimental evaluations and experimental data analysis. Therefore, all activities during the life-cycle of a winter wheat variety must be optimized in order to achieve economic success. Co-operation in breeding, VCU testing and production, subsequently also with processors of wheat and, above all, the acceptance of consumers is necessary. Based on own experience a main test site which requests high ecological adaptation should be chosen, with climatic conditions that provide broad information and with low to medium soil fertility. The variation of soil fertility at one trial site allows to optimize the selection of desired genotypes.

Suitable field trials at suitable test sites have to be used from the crossing to the deletion of a variety. Statistical methods have to be applied so that the 'genotype' can be separated from the 'phenotype'. The example of the varieties 'Capo', 'Georg' and 'Josef' demonstrated that these three varieties had favourable 'continental' genes in their pedigree, which led to their successful selection and cultivation in Central and Eastern European production regions. The described traditional way of breeding could be nowadays supported by genomic tools in order to select preferred genotypes more quickly but it should be not replaced by modern technologies.

Production-specific small plot trials should be carried out in parallel from the first VCU test year onwards as a farmer wishes to obtain as much information as possible about production characteristics of a newly released variety, which is still unknown in practice. It is recommended that wheat breeders include already during the individual selection steps at their trial sites long-tested check varieties so that the best lines can be clearly identified and put into the VCU testing.

At present both breeders and farmers are mostly not interacting with each other as the breeding activities of a company are largely independent of the seed production. Variety-specific recommendations of a breeders mostly relates only to the results of the VCU test. It should be noted that these results can be biased due to favourable environmental influences during the test period. Thus, the farmer is informed insufficiently as the VCU results provide no site- and variety-specific management recommendations. It is necessary to provide the best site- and variety-specific management recommendations to the farmer already at a variety's registration. Therefore, it is necessary to select suitable test sites, N-fertilization regimes, fungicide and growth regulator applications and seeding rates, both for organic and conventional production.

Both breeding and production should have all the results and information available through their trial sites network. Thereby, the official VCU test results are supplemented by the results of own production-related small plot trials. Thereby it will be possible to register significantly fewer varieties with only a low or no impact after their listing. As a result, the breeding progress in winter wheat should increase significantly each year.

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