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Nachruf: Peter Ruckebauer (1939-2019)

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Peter Ruckebauer wurde am 13. April 1939 in Wiener Neustadt geboren, wo er auch seine Pflichtschulzeit absolvierte. Die Matura legte Ruckebauer 1960 mit Auszeichnung an der Höheren Landwirtschaftlichen Bundeslehranstalt Francisco Josephinum in Wieselburg ab. Nach dem Präsenzdienst folgte von 1961 bis 1965 das Studium der Landwirtschaft an der damaligen Hochschule für Bodenkultur. Ab 1965 war Ruckebauer Wissenschaftler am Institut für Pflanzenbau und Pflanzenzüchtung und es folgte 1967 die Promotion und 1976 die Habilitation. Beide Arbeiten stellte er auch in Gumpenstein vor, 1966 seine Dissertation zu sortenspezifischen Wurzel/Sproßverhältnissen bei Getreide, 1976 seine Habilitation zur Erfassung von komplexen Eigenschaften mittels Cluster-Analyse. In dieser Zeit war es Ruckebauer auch gelungen, Getreideproben, die 1877 von Friedrich Haberlandt an der BOKU eingelagert wurden, wieder zum Keimen zu bringen. Die entsprechenden Weizen-, Gersten- und Hafermuster sind heute in der Genbank in Linz eingelagert und wertvolle Zeugen für die Leistung der Pflanzenzüchtung in den letzten 150 Jahren.

1983 wurde Ruckebauer an die Universität Hohenheim als Nachfolger von Wolfgang Schnell berufen. Auch während seiner Hohenheimer Zeit blieb Ruckebauer der Gumpensteiner Züchtertagung treu und er vollzog während dieser Zeit einen Wandel zum „Wissenschaftsmanager“. Nun waren es seine Studenten und jüngeren Kollegen, die die Tagung mit Vorträgen bereicherten.

Im Oktober 1989 wurde Ruckebauer nach Wien zurückberufen und sogleich machte er sich an die Arbeit, einerseits um das „verstaubte“ Institut für Pflanzenbau und Pflanzenzüchtung auszubauen und zu modernisieren, andererseits um das Interuniversitäre Forschungsinstitut für Agrarbiotechnologie (IFA) in Tulln zu realisieren. Hier baute Ruckebauer die erfolgreiche *Fusarium*-Resistenzforschung auf. Bis zu seiner Emeritierung 2005 leitete Ruckebauer sowohl den Lehrstuhl für Pflanzenzüchtung als auch das IFA-Tulln.

Drei Jahre nach seiner Emeritierung erschien Ruckebauers letzte wissenschaftliche Arbeit, in der ein Thema behandelt wurde, das auch im Mittelpunkt seines Vortrages bei der 33. Züchtertagung 1982 stand: die Indexselektion und die Nutzung von Komplexwerten zur Selektion. Im Jahr 2009 verabschiedete sich Ruckebauer in einer emotionalen Abschiedsrede von der Gumpensteiner Züchtergemeinschaft. Von 1966, damals als vortragender Dissertant,



bis zu seiner Abschiedsrede 2009 hat Peter Ruckebauer mit einer einzigen Ausnahme (1970 absolvierte er einen Forschungsaufenthalt am damaligen Plant Breeding Institute in Cambridge) an allen Züchtertagungen in Gumpenstein teilgenommen. Von 1991 bis 2007 war er hauptverantwortlich für die wissenschaftliche Organisation dieser Tagung und es war ihm immer ein Anliegen an einem „unmöglichen Ort zu einem unmöglichen Zeitpunkt“ Wissenschaft und Praxis zusammenzubringen. Der österreichischen Pflanzenzüchtung war Ruckebauer auch als Obmann der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs von 1999 bis 2003 und als wissenschaftliches Mitglied der Zuchtbuch- bzw. Sortenzulassungskommission von 1989 bis 2005 eng verbunden. Auch als praktischer Pflanzenzüchter reüssierte Ruckebauer: 1983 wurde eine seiner Sommerdurum-Kreuzungen als Sorte 'Unidur' in das „Zuchtbuch für Kulturpflanzen“ eingetragen.



Einweihung des Peter Ruckebauer-Hörsaals am IFA-Tulln anlässlich der 20-Jahr-Feier am 18. September 2014: Rudolf Krska (Wissenschaftlicher Leiter des IFA-Tulln), Hubert Hasenauer (Vorsitzender des Senats der BOKU), Martin Gerzabek (Rektor der BOKU), Peter Ruckebauer, Werner Biffel (Vorsitzender des Universitätsrates der BOKU), Erwin Pröll (Landeshauptmann von Niederösterreich) und Peter Eisenschek (Bürgermeister von Tulln) (v.l.n.r.)

Ruckebauers Verdienste um die heimische Pflanzenzüchtung und Landwirtschaft wurden 2005 mit der Verleihung des Hans-Kudlich-Preises für Verdienste um die Landwirtschaft Österreichs, sowie dem Silbernen Komturkreuz des Ehrenzeichens für Verdienste um das Bundesland Niederösterreich ausgezeichnet. Beim 19. Generalkongress der Europäischen Gesellschaft für Züchtungsforschung (EUCARPIA) wurde Ruckebauer zum Ehrenmitglied dieser Vereinigung ernannt.

Peter Ruckebauer verstarb am 14. April 2020, einen Tag nach Vollendung seines 80. Lebensjahres.

Ausgewählte Publikationen

Mardi M, Buerstmayr H, Ghareyazie B, Lemmens M, Moshrefzadeh N, Ruckebauer P (2004) Combining ability analysis of resistance to head blight caused by *Fusarium graminearum* in spring wheat. *Euphytica* 139: 45-50. DOI: 10.1007/s10681-004-2035-4

Ruckebauer P (1966) Sortenspezifische Wurzel-Sproßverhältnisse bei Getreide in Nährlösungen. 17. Arbeitstagung der Arbeitsgemeinschaft der Saatzuchtler, 23.-25. Nov., Gumpenstein, pp 193-215.

Ruckebauer P (1971) Keimfähiger Winterweizen aus dem Jahre 1877 - Beobachtungen und Versuche. *Bodenkultur* 22: 372-386.

Ruckebauer P (1976) Cluster-Analysen und ihre Möglichkeiten zur Erfassung von Komplexeigenschaften in der Getreidezüchtung. 27. Arbeitstagung der Arbeitsgemeinschaft der Saatzuchtler, 23.-25. Nov., Gumpenstein, pp 157-168.

Ruckebauer P (1982) Grundlegende Aspekte der Anwendung von Indizes in der Pflanzenzüchtung. 33. Arbeitstagung der Arbeitsgemeinschaft der Saatzuchtler, 23.-25. Nov., Gumpenstein, pp 157-168.

Ruckebauer P, Steiner AM (1995) Die agronomischen Eigenschaften des Nachbaues eines 110 Jahre alten Hafers - die Wiener Probe von 1877. *Bodenkultur* 46: 293-302

Schulze A, Steiner AM, Ruckebauer P (1994) Variability of an Austro-Hungarian landrace of barley (*Hordeum vulgare* L.) - Electrophoretic analysis of the hordeins of the Vienna sample of 1877. *Plant Var Seed* 7: 193-197.

Sölkner J, Gausgruber H, Okeyo AM, Ruckebauer P, Wurzing M (2008) Breeding objectives and the relative importance of traits in plant and animal breeding: A comparative review. *Euphytica* 161: 273-282. DOI: 10.1007/s10681-007-9507-2

Steiner AM, Ruckebauer P (1995) Germination of 110-year-old cereal and weed seeds, the Vienna Sample of 1877. Verification of effective ultra-dry storage at ambient temperature. *Seed Sci Res* 5: 195-199. DOI: 10.1017/S0960258500002853

Weiterführende Literatur

Steiner AM (1999) Zur Geschichte der Wiener Pflanzenzüchtung: Haberlandt, Fruwirth, Ruckebauer - Saat und Ernte. *Bodenkultur* 50: 203-210. [<https://diebodenkultur.boku.ac.at/volltexte/band-50/heft-3/steiner.pdf>]

Vollmann J, Gausgruber H, Lemmens M, Bürstmayr H (2019) Peter Ruckebauer (1939-2019) - Ein wissenschaftlicher Nachruf. *Bodenkultur* 70: 125-129. DOI: 10.2478/boku-2019-0009

Comparing the breeding progress in winter wheat between organic and conventional management in Germany

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Plant breeding makes a significant contribution to increasing and maintenance of yield and thus the efficiency of crop production. For winter wheat in Germany under conventional management, previous studies have found breeding progress in winter wheat of between 0.25 and 0.8 dt ha⁻¹ per year (Laidig *et al.* 2014). While conventional on-farm yields in Germany still increase linearly with a similar rate (Laidig *et al.* 2014), organic yields have remained constant since around 25 years. An important question regarding breeding progress is whether it depends on the intensity of cultivation. With regard to the stagnation of winter wheat yields under organic management, we investigated if the realization of breeding progress depends on the cultivation intensity, particularly if it differs between conventional and organic management.

Yield data of winter wheat were extracted from publicly available reports of the conventional and organic networks for variety testing and recommendation in Germany (Landessortenversuche) for the years 2001 to 2017. Data were analysed using a mixed model in which genetic progress (breeding progress) can be separated from non-genetic progress (agronomic and climate-related) by separate regression terms on the year of registration and year of testing, respectively (Laidig *et al.* 2014). Only varieties tested for at least 3 years were included. In total, the data comprised about 2400 trials (conventional and organic), which corresponds to about 140 trials per year. The average yield level of the conventional trials was 95 dt/ha and 51 dt/ha under organic management.

Using the mixed-model method, the genetic progress across over all variety classes in the conventional trials with regular plant protection was estimated to 0.39 dt ha⁻¹ per year ($P < 0.001$, t -test) and

0.73 dt ha⁻¹ per year ($P < 0.001$) under reduced intensity without fungicides and growth regulators (Table 1). These estimates are in agreement with previous estimates (Laidig *et al.* 2014). In the trials under organic management, this estimate was 0.04 dt ha⁻¹ per year and was not significantly ($P > 0.05$) different from zero. The observation of absent breeding progress under organic management was also observed by Hildermann *et al.* (2009), who tested a set of winter wheat varieties under conventional and organic management and detected breeding progress only under conventional management. While estimates of breeding progress were similar in all quality classes in the conventional network with regular plant protection, in the organic network there was significant breeding progress only in quality class A.

A possible reason that organic management does not benefit from breeding progress could be that breeding is not successful towards better disease resistances. However, the higher breeding progress in conventional management without fungicides suggests, that breeding has been very efficient towards increased disease resistance. Breeding for resistance towards particular diseases that are problematic in organic farming, but not in conventional farming (particularly bunts and smuts), because they can be sufficiently controlled through chemical plant protection, is still of particular importance.

Furthermore, we found a strong correlation of the performance of 66 varieties, which were tested in both networks ($r = 0.78^{***}$ under optimal and $r = 0.76^{***}$ under reduced intensity, respectively). This suggests that breeding and testing under conventional management is - at least partly - also representative for the per-

Table 1: Estimates of breeding progress (b) for the organic and conventional network with regular plant protection for different quality classes and over all quality classes.

Quality class	Organic				Conventional				
	Varieties	Trials	Yield (dt ha ⁻¹)	b (dt ha ⁻¹ y ⁻¹)	Varieties	Trials	Yield (dt ha ⁻¹)	b (dt ha ⁻¹ y ⁻¹)	
E	63	477	49.1	0.06 ns	26	1322	90.1	0.40 ***	
A	36	473	52.2	0.38 ***	76	1904	94.0	0.35 ***	
B	13	266	53.3	-0.06 ns	67	1900	96.8	0.39 ***	
C	4	221	60.0	0.21 ns	23	1720	98.8	0.42 ***	
Overall	116	477	50.6	0.09 ns	192	1905	94.9	0.39 ***	

formance under organic conditions. Despite this correlation, separate evaluation of varieties under organic conditions might still be advantageous for traits like weed suppression and lodging tolerance, as these traits are of less importance in conventional farming. Another possible explanation for the unobserved breeding progress under organic management could be the yield limitation by available nitrogen. This argument is supported by the fact that organic trials with legumes as preceding crops (mainly grass-clover ley) show a higher breeding progress on average than trials with non-legumes as preceding crops (0.11 vs 0.02 dt ha⁻¹ per year).

Based on these results, we argue that plant breeding is probably only fully efficient if other resources are sufficiently available (particularly nitrogen). Plant breeding and agronomic management are thus intertwined, and for each management system, the yield limiting key factors need to be identified. While plant breeding is very efficient under conventional management without fungicide control, in organic farming the key factor is rather to increase the availability of nitrogen through better nitrogen management.

Keywords

Genetic progress · mixed model · nitrogen · *Triticum aestivum*

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References

- Hildermann I, Thommen A, Dubois D, Boller T, Wiemken A, Mäder P (2009) Yield and baking quality of winter wheat cultivars in different farming systems of the DOK long-term trial. *J Sci Food Agric* 89: 2477-2491. DOI: 10.1002/jsfa.3750
- Laidig F, Piepho H-P, Drobek T, Meyer U (2014) Genetic and non-genetic long-term trends of 12 different crops in German official variety performance trials and on-farm yield trends. *Theor Appl Genet* 127: 2599-2617. DOI: 10.1007/s00122-014-2402-z

A meta-analysis comparing the temporal yield stability between organic and conventional farming - communication and reception of a complex finding

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In a meta-analysis, based on the published data of Ponisio *et al.* (2015), we compared the temporal yield stability of organic and conventional farming. We used two different parameters to assess stability: (i) the standard deviation as a measure of absolute stability, and (ii) the coefficient of variation as a measure of relative stability. Absolute stability measures the variation of yields over time, whereas relative stability is corrected for the yield level, by dividing absolute stability by the mean yield. In the meta-analysis, for each pair of observations the ratio of the stability of both systems was calculated. Overall, 165 comparisons were included, based on at least 4 years of observation.

Overall, the yield in organic farming was 16% lower than in conventional farming (Figure 1). While this yield gap is considerably greater in Western Europe (e.g. around 50% in cereals in Germany), the observed yield gap in our study is due to the global origin of the data, including countries, where conventional management is less intensive. We found no significant difference in absolute stability. However, relative stability was 15% lower in organic far-

ming. As relative stability is corrected for the difference in yield, the difference in relative stability is mainly due to the higher yield in conventional farming.

As the interpretation of the result depends on the stability parameter (absolute vs. relative stability), it first appeared difficult to communicate the findings in a press release. After all, we used exactly this problem as the title of the press release (Figure 2). The press-release was picked up on Twitter and by many agricultural magazines, mainly on their online page. The difference between absolute and relative stability was mentioned in some articles. However, there were also some articles referring only to the difference in relative stability, without mentioning, that the difference was found for relative stability, but citing it only 'stability'.

As the interpretation is depending on the parameter, we aimed to communicate the results in a neutral way, and even used the complexity of interpretation as the title. However, the way of reproduction through the different press organs was rather diverse.

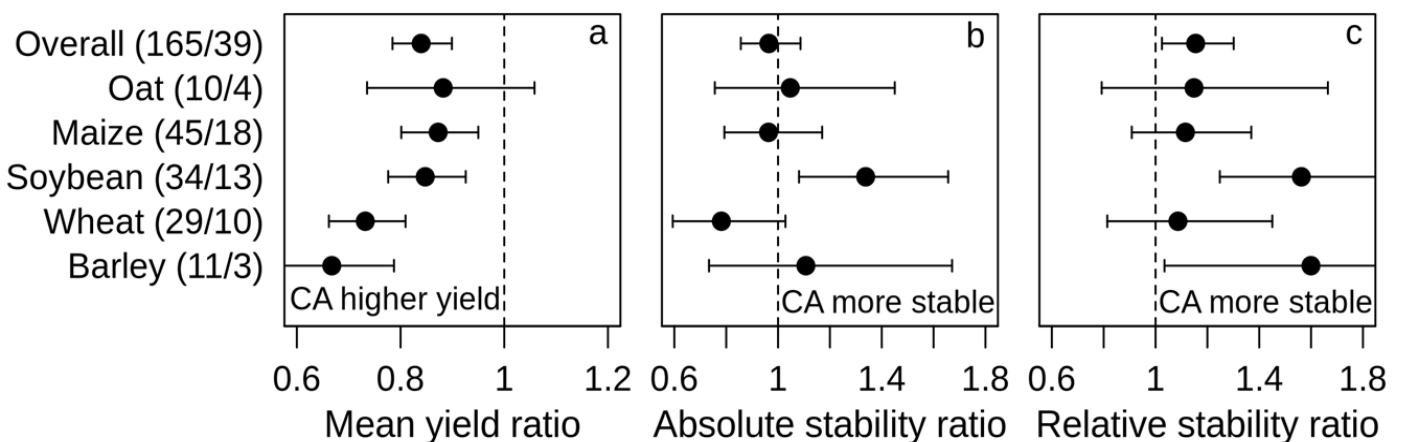


Figure 1: Grain yield (a), absolute stability (b) and relative stability (c) ratios between organic and conventional agriculture (CA). Numbers in parentheses denote the number of observations and studies, respectively. A ratio of 1 means no difference between the two management systems while values <1 indicate higher grain yields for CA and a stability measure ratio >1 indicates greater stability for CA. Dots and whiskers represent mean values and 95% confidence intervals, respectively. The effect is significant, if the confidence interval does not include the value of 1.

Meta-analysis of yield stability compares organic and conventional agriculture

The distinction between absolute and relative stability

SIMILAR YIELD FLUCTUATIONS, BUT DIFFERENT YIELD LEVELS

However, the variations in yields were very similar over the years in both cultivation systems. "While this may sound reassuring, it does have a catch," says Samuel Knapp. "Because the absolute yield variations are similar, the fluctuations in the lower yields of organic farming have a greater impact. The so-called relative yield stability is therefore lower in organic farming."

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Figure 2: Press release of the article by Knapp & van der Heijden (2018) (left) and titles of headings of articles published by agricultural magazines following the press release (right).

Keywords

Barley · conventional agriculture · maize · oat · organic agriculture · soybean · wheat · yield gap · yield stability

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References

- Knapp S, van der Heijden MGA (2018) A global meta-analysis of yield stability in organic and conservation agriculture. *Nat Commun* 9: 3632. DOI: 10.1038/s41467-018-05956-1
- Ponisio LC, M'Gonigle LK, Mace KC, Palomino J, de Valpine P, Kremen C (2015) Diversification practices reduce organic to conventional yield gap. *Proc R Soc Lond B Biol Sci* 282: 20141396. DOI: 10.1098/rspb.2014.1396

Breeding methodologies for mixed cropping using barley-pea mixtures as a model system

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Mixed cropping is the simultaneous cultivation of different crop species in the same field at the same time. Especially combinations of legume with non-legume plants provide agronomic advantages, such as increased productivity and higher yield stability under low-inputs conditions. In mixed cropping, choosing the right cultivars is crucial for the performance of the crop, as shown for e.g. maize with bean by Hoppe (2016). As performance in pure stand can strongly diverge from performance in mixture, estimating the ability of a cultivar to be mixed with another crop is therefore of utmost importance. For this purpose, concepts of general and specific combining ability from hybrid breeding have been adapted to cultivar and crop mixtures. Thus, these effects are called general mixing ability (GMA) and specific mixing ability (SMA). The harvest of most interspecific mixtures can be separated due to different grain sizes. This fact allows to access valuable information about competition and facilitation among mixing partners, leading to deviating fraction yields. Until now, statistical developments have neglected to mobilize the additional information provided by these separated harvest lots. The concept of producer- and associate-effects (abbreviated *Pr* and *As*) describes interactions between varieties in this context. The *Pr* is the average performance of a cultivar grown in mixture with a companion crop, whereas *As* is the average effect of a cultivar on the respecti-

ve mixing partner. However, in the past the *Pr/As*-concept has only been applied to variety mixtures of the same species, sown in alternate row trials. We used the fraction yields of a spring-pea (*Pisum sativum* L.) and spring-barley (*Hordeum vulgare* L.) mixed cropping experiment to determine *Pr* and *As* of different pea genotypes. The additional information provided by this approach is biologically more informative than GMA/SMA estimates, since it better reflects competition and facilitation occurring between different cultivars of the two crop-species.

Plant material comprised 28 pea- (plus 4 mixtures) and 7 barley- (plus 1 mixture) cultivars. The genotypes were chosen in a way to maximise morphological diversity and origin (breeding programs). A subset of all possible combinations was sown: 56 bi-specific pea-barley mixtures were arranged in an incomplete factorial design (Figure 1). Mixtures and pure stands were sown in 7.5 m² plots with two repetitions at two locations in Switzerland. Harvested grains were separated into pea and barley fractions. Variance components for both the GMA/SMA and the *Pr/As* model were estimated within a mixed model framework with best linear unbiased prediction. GMA of pea cultivars, SMA (interaction of pea cultivar with barley cultivar) and the error term were set as random variables. The random effects were assumed of having a mean

	peas																																	
	P01	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23	P24	P25	P26	P27	P28	P29	P30	P31	P32		
barleys	No barley (pure stand pea)																																	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
	No pea (pure stand barley)																																	
B1	1	1			1					1				1					1			1				1			1					
B2	1	1				1			1						1		1					1						1		1				
B3	1			1	1				1					1						1	1				1						1			
B4	1		1					1				1				1	1						1					1				1		
B5	1			1		1					1		1					1					1				1						1	
B6	1	1				1					1		1							1	1					1					1			
B7	1	1					1			1					1		1							1		1						1		1
B8	1		1					1				1			1				1					1			1		1					

Figure 1: Incomplete factorial design with 8 barley pure stands (7 cultivars and 1 variety mixture), 32 pea pure stands (28 cultivars and 4 variety mixtures), and 56 bi-specific mixtures of those.

of 0 and being normally distributed. In analogy, Pr and As effects were estimated with the pea and barley component yields as dependent variables, respectively. Potential functional traits, such as early vigor of pea, were measured and evaluated using correlation analysis to relate them to GMA, Pr and As effects. When prerequisites for parametric test procedures were not fulfilled, non-parametric tests (e.g. Spearman rank-correlation) were applied.

The proportion of GMA variance of pea is the variance in mixture yield explained by the presence of a given pea cultivar in mixture. We found this variance to be predominant over SMA variance (the variance due to interaction of pea and barley cultivars). Our analyses yielded variance components of GMA pea $\approx 40\%$, SMA $\approx 10\%$, residual $\approx 50\%$. There was a significant negative correlation between the pea Pr and its As with Spearman's $r_s = -0.47$. However, few individual pea genotypes were found with positive Pr and positive As . The As of pea were correlated over locations ($R^2=0.48$). The GMA of pea was not significantly correlated with early vigor of pea ($r_s = 0.21$), whereas As of pea had a significant negative correlation with this trait ($r_s = -0.36$).

The GMA approach, based on the testcross methodology from hybrid breeding is a valuable tool to determine mixing ability in pea-barley mixtures. This potential is further pronounced by our finding that pea GMA variance is predominant over SMA variance, indicating the potential for breeding for mixed cropping. The GMA approach can be extended using the Pr/As concept for understanding trait influences on mixture behaviour. We observe a negative correlation between Pr and As effects, indicating a trade-off between a cultivar's performance and its companion-crop's performance as observed also by Forst (2018) for wheat cultivar mixtures. However, our data suggests room for genetic improvement, e.g. by selecting deviating genotypes with both positive Pr and As effects. As effects were correlated over locations, indicating an underlying heritable component. Early vigor of pea was not correlated with GMA, however, it was significantly negatively correlated with pea's As effect, i.e. high early vigor had a negative effect on barley yield. This indicates the surplus of precision and infor-

mation on trait-performance relationships that the Pr/As -concept gives compared to the GMA concept. The results allow us to (i) seize the effects of cultivar choice in the performance of crop mixtures, and (ii) to propose breeding schemes and experimental designs for improving both crops with respect to pea-barley mixtures.

Keywords

General mixing ability · *Hordeum vulgare* · intercropping · *Pisum sativum*

Acknowledgements

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References

- Bedoussac L, Journet E-P, Hauggaard-Nielsen H, Naudin C, Corre-Hellou G, et al. (2015) Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. *Agron Sust Dev* 35: 911–935. DOI: 10.1007/s13593-014-0277-7
- Federer W (1993) Statistical design and analysis for intercropping experiments, Vol I: Two crops. Springer, New York. DOI: 10.1007/978-1-4613-9305-4
- Forst E (2018) Développement de méthodes d'estimation de l'aptitude au mélange pour la prédiction des performances et la sélection de mélanges variétaux chez le blé tendre et co-conception d'idéotypes de mélanges adaptés à l'agriculture biologique. Université Paris Saclay. [<https://tel.archives-ouvertes.fr/tel-02114929/document>; accessed April 16, 2020]
- Hoppe C (2016) Entwicklung von Energiemaissorten für die Mischkultur mit Stangenbohnen. PhD Thesis, Niedersächsische Staats- und Universitätsbibliothek Göttingen. [<http://hdl.handle.net/11858/00-1735-0000-0028-87D0-F>; accessed April 16, 2020]

Resistance breeding - Cornerstone for future plant protection strategies

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Breeding for resistance to biotic stress in cereals is of prime importance to (i) avoid yield losses, (ii) ensure a consumer and environmental friendly production, and (iii) protect biodiversity. Resistance breeding will gain more importance in the EU due to a loss of fungicides and insecticides resulting from political restrictions. It is estimated that the global average yield loss due to diseases in wheat and barley is >20%. In Germany, wheat breeding in the last 50 years contributed significantly to sustainability-related traits such as nitrogen use efficiency or disease resistance. The economic impact of diseases on wheat production and the ability of pathogens to generate new virulent pathotypes in a short period of time necessitates the search for new resistance genes and in parallel the development of tools for their efficient deployment in breeding programs. Efficient resistance breeding requires first the identification of genetic variation for resistance within the gene pool and the development of effective screening methods, followed by the genetic analysis of resistance and the development of molecular markers. The availability of dense marker maps, high throughput genotyping platforms, physical maps and genome sequences as well as of genome wide association studies (GWAS) facilitates an enhanced marker development and isolation of resistance genes thereby leading to a deeper understanding of resistance. The introgression of resistance genes by marker-assisted backcrossing and their pyramiding is nowadays a major component of the plant breeder's toolbox. In addition, knowledge on resistance gene sequences will facilitate efficient allele mining and the targeted editing of the respective alleles by e.g. CRISPR/Cas9. This will lead to a more direct use of plant genetic resources in wheat and barley breeding thereby broadening the genetic basis of resistance and securing an environmental friendly and productive cereal production in the face of climate change and loss of insecticides and fungicides.

Keywords

Allele mining · cereal · economic loss · gene editing · gene pyramiding · genetic map · molecular marker · sustainable production

References

- Cantalapiedra CP, Contreras-Moreira B, Silvar C, Perovic D, Ordon F, *et al.* (2016) A cluster of nucleotide-binding site-leucine-rich repeat genes resides in a barley powdery mildew resistance quantitative trait loci on 7HL. *Plant Genome* 9. DOI: 10.3835/plantgenome2015.10.0101.
- Fazlikhani L, Keilwagen J, Kopahnke D, Deising H, Ordon F, Perovic D (2019) High resolution mapping of *Rph*_{MBR1012} conferring resistance to *Puccinia hordei* in barley (*Hordeum vulgare* L.). *Front Plant Sci* 10: 640. DOI: 10.3389/fpls.2019.00640
- Karlstedt F, Kopahnke D, Perovic D, Jacobi A, Pillen K, Ordon F (2019) Mapping of quantitative trait loci (QTL) for resistance against *Zymoseptoria tritici* in the winter spelt wheat accession HTRI1410 (*Triticum aestivum* subsp. *spelta*). *Euphytica* 215: 108. DOI: 10.1007/s10681-019-2432-3
- Kassa MT, Haas S, Schliephake E, Lewis C, You FM, *et al.* (2016) A saturated SNP linkage map for the orange wheat blossom midge resistance *Sm1*. *Theor Appl Genet* 129: 1507-1517. DOI: 10.1007/s00122-016-2720-4
- König J, Kopahnke D, Steffenson BJ, Przulj N, Romeis T, *et al.* (2012) Genetic mapping of a leaf rust resistance gene in the former Yugoslavian barley landrace MBR1012. *Mol Breed* 30: 1253-1264. DOI: 10.1007/s11032-012-9712-0
- Novakazi F, Afanasenko O, Anisimova A, Platz GJ, Snowdon R, *et al.* (2019) Genetic analysis of a worldwide barley collection for resistance to net form of net blotch disease (*Pyrenophora teres* f. *teres*). *Theor Appl Genet* 132: 2633-2650. DOI: 10.1007/s00122-019-03378-1
- Silvar C, Perovic D, Scholz U, Casas AM, Igartua E, Ordon F (2012) Fine mapping and comparative genomics integration of two quantitative trait loci controlling resistance to powdery mildew in a Spanish barley landrace. *Theor Appl Genet* 124: 49-62. DOI: 10.1007/s00122-011-1686-5
- Silvar C, Perovic D, Nussbaumer T, Spannagl M, Usadel B, *et al.* (2013) Towards positional isolation of three quantitative trait loci conferring resistance to powdery mildew in two Spanish barley landraces. *PLoS One* 8: e67336. DOI: 10.1371/journal.pone.0067336
- Verchot J (2012) Cellular chaperones and folding enzymes are vital contributors to membrane bound replication and movement complexes during plant RNA virus infection. *Front Plant Sci* 3: 275. DOI: 10.3389/fpls.2012.00275
- Voss-Fels KP, Stahl A, Wittkop B, Lichthardt C, Nagler S, *et al.* (2019) Breeding improves wheat productivity under contrasting agrochemical input levels. *Nature Plants* 5: 706-714. DOI: 10.1038/s41477-019-0445-5
- Yang P, Lüpken T, Habekuss A, Hensel G, Steuernagel B, *et al.* (2014) *PROTEIN DISULFIDE ISOMERASE LIKE 5-1* is a susceptibility factor to plant viruses. *Proc Natl Acad Sci* 111: 2104-2109. DOI: 10.1073/pnas.1320362111

Importance of biotic and abiotic stress resistance in sustainable potato production

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Abstract

In view of climate change and the associated increasing frequency of weather extremes, profitable potato production requires intensive inputs (e.g. fertilisation, plant protection, irrigation) and the cultivation of varieties with a broad range of resistance to biotic and abiotic stresses. Despite the overall adaptability of the crop, varieties may significantly differ in their reaction to environmental factors such as the incidence of pathogens, pests, heat, drought or irregular water supply. Due to specific genotype by environment interactions, the ecological conditions of the site where the breeding programme takes place may have high impact on the success of released varieties under stress conditions. Here we report on the effect of complex (multiple) resistance of eight Hungarian potato varieties on yield and quality in comparison to check cultivars during their official VCU and post-registration trials. All tested varieties performed better than the checks in each test year with respect to the occurrence of tuber disorders, marketable yield and major virus diseases (PLRV, PVY). In general, the new varieties showed a lower susceptibility to common scab, late blight and tuber rotting pathogens. Marketable yield was similar to the checks in the first VCU year, but outperformed the checks in subsequent years, demonstrating that biotic and abiotic stress resistances derived from wild species can be combined with yield and quality at variety level. The cultivation of such varieties can significantly improve the security of potato production and increase its profitability under conventional, integrated or especially organic farming.

Keywords

Common scab · late blight · multiple resistance · organic farming · *Solanum tuberosum* · tuber disorder · virus disease

Introduction

Potato is a sensitive crop both to biotic and abiotic stresses (van Loon 1981), being susceptible to many pathogens and pests and having a weak root system. Potato diseases of major economic importance are potato virus Y (PVY, especially the strain PVYNTN), potato leafroll virus (PLRV), late blight (*Phytophthora infestans*) and common scab (*Streptomyces scabies*). Potato tubers are especially sensitive to heat and drought stress. The stress sensitivity of

a variety results in yield decrease (lower tuber number and weight/plant) and quality loss (tuber malformations, internal defects, higher sugar or alkaloid content etc.) (Vayda 1994, Levy & Veilleux 2007). Virus diseases are responsible for the so-called potato degeneration, a biological value-deterioration process which results in considerable quantitative and qualitative yield loss in case of cultivation of farm-saved seeds.

The EU Directive 2009/128/EC on the sustainable use of pesticides summarizes the eight general principles of Integrated Pest Management (IPM). One of the most powerful options of sustainable disease management is the extended use of resistant cultivars, especially those carrying multiple resistance or tolerance to biotic and abiotic stresses. Potato breeders have paid attention to improve the stress resistance of new varieties during the last decades in Hungary and worldwide (Sárvári 1967, Ross 1986, Lönhárd *et al.* 2002, Polgár *et al.* 2010, Bradshaw 2016). However, it is a rather difficult, time and effort consuming process to develop multiple disease resistant/stress tolerant varieties with also high tuber yield and quality, respectively (Leppik 1970).

The Potato Research Centre at Keszthely deals with resistance breeding since 1960. One of its major duties is the breeding of varieties suitable for Central European agro-ecological conditions due to their resistance against major pests, pathogens and abiotic stresses. The applied breeding strategy is a complex approach based on the utilisation of exotic germplasm of wild relatives of cultivated potato. Major techniques applied are: somatic hybridisation to overcome sexual crossing barriers, negative and positive selection of stress sensitive/resistant genotypes under natural stress conditions or after artificial infections with pathogens and pests, application of DNA markers linked to certain resistance genes, application of a complex parental line evaluation system to predict the breeding value of each cross combinations (Polgár *et al.* 2016). Here we report on the results regarding biotic and abiotic stress tolerance of eight Hungarian potato cultivars originating from the breeding programme at Keszthely during their VCU testing.

Material and Methods

All VCU trials were carried out by the National Food Chain Safety Office of Hungary (NÉBIH) according to its standardised testing protocol at 4-6 locations with 4 replications per site and 56 plants per replicate. The trials were set up for 2 or 3 subsequent years:

'Hópehely' (1993-1996), 'White Lady' (1993-1994), 'Balatoni rózsza' (2005-2006), 'Démon' (2007-2008), 'Arany Chipke' (2011-2012), 'Botond' (2013-2014) and 'Basa' (2015-2016). Post-registration trials were conducted for 'Hópehely' (1997-1998) and 'White Lady' (1995-1998).

The following check varieties were used: the Dutch cvs. 'Cleopatra' (early to very early maturity group) and 'Desirée' (mid-early to mid-late maturity group) for table potatoes, the old Hungarian cv. 'Somogyi sárga kifli' for salad-type potatoes, and the Dutch cv. 'Lady Rosetta' for chips potatoes.

Virus resistance

Data (% infected plants) were collected based on visual evaluation of the main symptoms (*i.e.* leaf-rolling caused by secondary infection, vein necrosis and/or ink-spotting on the leaves, mild, rugose and severe mosaic) at flowering. ELISA tests were performed from randomly collected leaf samples in every symptomatic category, including the major potato viruses (PLRV, PVY, PVM, PVA, PVX, PVS) to confirm correspondence of visible symptoms with detectable pathogen(s). Evaluation (% infected tubers) of net necrosis (caused by PLRV) and necrotic ringspot disease (caused by PVYNTN) was done on randomly collected samples of 2×100 tubers per variety originated from elite and 1st generation.

Late blight

Testing for foliar resistance to *Phytophthora infestans* (% infected leaf area) was carried out in a whole-plant inoculation trial in a walk-in plastic tunnel using a complex domestic race of the pathogen at the Post-Control Station of NÉBIH in Monorierdő.

Common scab

Data (index of infection %) are based on the symptomatic evaluation of randomly collected samples of 2×100 tubers (elite + 1st generation) originating from VCU trials under natural infection of *Streptomyces scabies* after a storage period of 2 months.

Tuber rots

Data (% infected tubers) are based on the symptomatic evaluation of randomly collected samples of 2×100 tubers (elite + 1st generation) originating from VCU trials under natural infection with *Fusarium* spp., *P. infestans*, *Alternaria solani* and/or *Pectobacterium* spp. after a storage period of 2 months.

Physiological tuber disorders

Symptomatic evaluation of traits (*e.g.* malformation, secondary growth, tuber cracking, glassiness, internal rust spot) was done based on the incidence of disorders on randomly collected samples of 2×100 tubers (elite + 1st generation) originated from VCU trials after a storage period of 2 months. Symptoms were recorded as % affected tubers and/or index of % spotting/glassiness).

Results and discussion

Eight Hungarian potato varieties were evaluated during their VCU and post-registration trials. The varieties originated from a resistance breeding programme focusing on the combination of major resistance genes against viruses and other potato pathogens and pests (*e.g.* late blight, nematodes, wart, common scab) with high yielding potential and quality. All tested varieties carry efficient resistance to PVY and PVA, two to PVX, all have a high level of field resistance to PLRV, five of them are immune against

Table 1: Abiotic stress response of cvs. 'Hópehely' and 'White Lady' versus check cv. 'Desirée' in VCU and post-registration trials (in brackets). Mean values of elite and 1st generation tubers (2×100 tubers from VCU trials, 1993-1996) and elite generation of 1×100 tubers from the post-registration trials (1995-1998)

Year	Malformation (% tuber)			Secondary growth (% tuber)		
	Hópehely	White Lady	Desirée	Hópehely	White Lady	Desirée
1993	20.4	22.2	29.6	2.1	1.6	3.8
1994	20.7	30.0	38.0	0.7	1.2	4.0
1995	20.2	(23.4)	28.5 / (32.3)	0.8	(0.9)	2.6 / (2.2)
1996	22.2	(19.8)	38.0 / (38.0)	0.4	(0.8)	3.2 / (6.3)
1997	(14.8)	(12.0)	(26.3)	(1.3)	(0.3)	2.3
1998	(21.7)	(20.0)	(35.0)	(2.0)	(0.7)	4.7
Mean	20.0	21.2	32.6 / 33.2	1.2	0.9	3.4 / 3.9
Year	Tuber cracking (% tuber)			Internal rust spots (index of % spotting)		
	Hópehely	White Lady	Desirée	Hópehely	White Lady	Desirée
1993	0.4	0.4	2.1	0.0	0.0	0.0
1994	1.7	0.8	2.0	0.0	0.0	0.0
1995	2.5	(1.3)	1.1 / (0.5)	0.0	(0.0)	0.0 / (0.1)
1996	1.0	(1.5)	0.5 / (0.5)	0.0	(0.1)	0.0 / (0.0)
1997	(0.5)	(0.5)	0.5	(0.0)	(0.0)	0.0
1998	(2.0)	(3.0)	1.3	(0.0)	(0.0)	0.0
Mean	1.4	1.3	1.3 / 1.2	0.0	0.0	0.0 / 0.0

the infection of Ro1-4 cyst nematodes and potato wart pathotype D1, while showing moderate resistance to common scab (Polgar *et al.* 2016).

Tuber disorders

Genotypic reaction to abiotic stresses can be different. Sensitivity manifests mainly in tuber disorders such as shape malformation, secondary growth, cracking or internal rust spots. Table 1 summarizes the data obtained during VCU and post-registration trials for cvs. 'Hópehely' and 'White Lady'. Both varieties revealed significantly lower tuber disorders than the check cv. 'Desirée'. For 'Hópehely', the proportion of distorted tubers was 12% lower on the average of six years, while the rate of secondary growth was 79% lower compared to 'Desirée'. The incidence of tuber cracks and internal rust spots was negligible for both varieties. 'White Lady' showed a similar good performance, outperforming 'Desirée' with respect to the frequency of malformation and secondary growth, and being equal regarding cracked tubers and internal rust spots.

The results of the newly registered varieties for tuber defects are shown in Table 2. In total, all Hungarian varieties showed less problems concerning tuber quality than the check varieties of the

respective maturity and end-use groups. In terms of tuber shape stability, 'Démon' (91.5%), 'Basa' (91.4%) and 'Balaton rose' (88.4%) were the best performing genotypes in the 1st test year, while the check varieties 'Lady Rosetta' and 'Somogyi sárga kifli' showed a tuber shape stability of 81.2% and 53.5%, respectively. In the 2nd test year with replanted 1st year harvested tubers, again 'Basa' and 'Démon' produced the lowest number of distorted tubers (*i.e.* 10.6% and 15%, respectively), while the check varieties generally continued to deteriorate (*e.g.* 'Cleopatra' 55.6% and 'Desirée' 44.4%).

Regarding secondary growth, four varieties showed a negligible incidence of such tubers, *i.e.* 'Botond' (1.1%), 'Démon' (0.9%), 'Arany Chipke' (1.9%), and 'Basa' (1.5%). For check cvs. 'Cleopatra', 'Desirée', 'Lady Rosetta' and 'Somogyi sárga kifli' the incidence was 9.6%, 11.5%, 5.0% and 16.4%, respectively. The index for glassiness and internal rust spots was negligible for all varieties and test years. Over all tuber related traits it can be summarized that the eight new varieties significantly improved the marketability and external quality of the tubers (Figure 1). Compared to the check varieties, the characteristic tuber shape was stable across the test years despite varying weather conditions.

Table 2: Abiotic stress response of new and check potato varieties in two years of Hungarian VCU trials. (E = elite seed; e = 1st generation seed)

Cultivar	Generation	Malformation (%)		Secondary growth (%)		Tuber cracking (%)		Glassiness index (%)		Internal rust spots index (%)	
		1	2	1	2	1	2	1	2	1	2
Test year		1	2	1	2	1	2	1	2	1	2
Balaton rózsza	E	8.3	15.0	2.3	13.3	0.3	10.7	0.0	0.2	0.1	0.0
	e	-	17.7	-	12.0	-	5.3	-	0.2	-	0.0
Cleopatra	E	31.3	33.3	8.5	13.7	4.3	17.7	0.1	0.4	0.4	0.4
	e	-	43.3	-	18.3	-	16.7	-	0.4	-	0.4
Botond	E	11.0	17.5	0.4	1.8	0.4	1.3	0.0	0.1	0.1	0.0
	e	-	16.3	-	1.0	-	0.0	-	0.0	-	0.0
Cleopatra	E	33.0	53.8	1.6	9.8	0.2	3.5	0.1	0.2	0.9	0.2
	e	36.3	57.5	4.0	11.0	1.0	2.8	0.2	0.4	0.8	0.2
Démon	E	6.0	11.0	2.0	0.4	1.3	2.2	0.1	0.0	0.2	0.0
	e	-	15.0	-	0.2	-	1.6	-	0.0	-	0.0
Desirée	E	37.0	41.3	13.8	6.2	1.3	4.0	0.2	0.1	0.0	0.0
	e	-	47.5	-	6.2	-	3.2	-	0.1	-	0.0
Katica	E	11.0	24.0	3.5	14.3	2.0	4.7	0.1	0.5	0.0	0.0
	e	-	25.0	-	11.0	-	4.0	-	0.5	-	0.0
Desirée	E	27.0	31.7	8.5	20.7	1.5	6.0	0.1	0.4	0.0	0.1
	e	33.8	22.7	9.0	16.0	2.0	5.7	0.0	0.2	0.0	0.1
Arany Chipke	E	21.3	16.3	2.0	1.8	4.5	1.5	0.0	0.0	0.0	0.1
	e	-	13.8	-	1.8	-	2.0	-	0.1	-	0.0
Lady Rosetta	E	20.0	17.5	4.3	6.5	2.5	0.3	0.0	0.1	1.7	0.2
	e	-	20.0	-	4.3	-	4.5	-	0.0	-	0.0
Basa	E	11.0	6.2	3.4	0.6	0.6	0.0	0.1	0.1	0.0	0.0
	e	-	10.6	-	0.6	-	0.2	-	0.0	-	0.0
Somogyi sárga kifli	E	41.0	52.0	22.6	13.8	6.0	0.4	1.0	0.0	0.2	0.2
	e	-	33.0	-	12.8	-	0.6	-	0.0	-	0.2

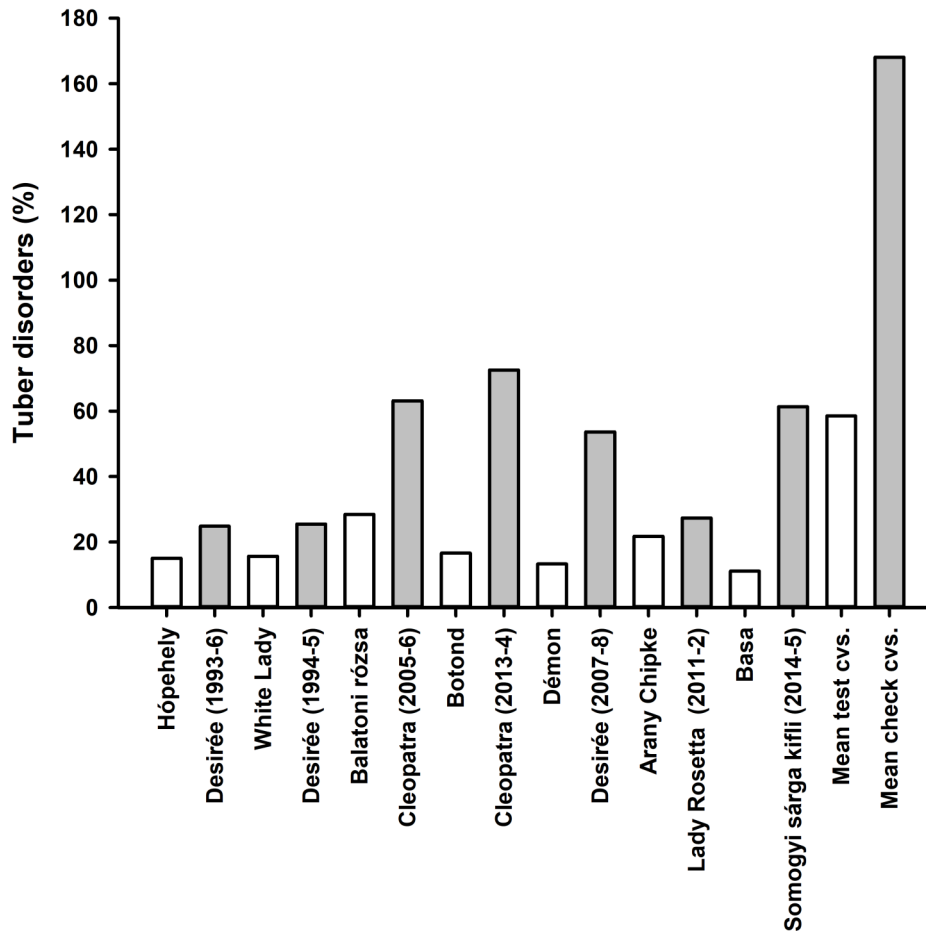


Figure 1: Pairwise comparisons of cumulated tuber disorders of new Hungarian varieties (white bars) versus their respective check cultivars (grey bars) in the particular variety registration (VCU) trial periods (indicated in brackets for the check cvs.).

Disease symptoms

Quantitative and qualitative loss in yield due to repeated replanting of seed potato is called degeneration. The main reason for this is the increase of virus-infected potato plants in the field. The main causes of degeneration are PVY and PLRV, which cause epidemics every year under Hungarian growing conditions. The only effective mitigation strategy is the cultivation of resistant varieties. Late blight is the most devastating fungal disease of potatoes. Although it is possible to control late blight by the use of fungicides, there is an increasing demand for resistant varieties due to food safety and environmental aspects. In Tables 3 and 4 the results of the new varieties during their VCU and post-registration trials are presented.

The results clearly show that the virus resistance genes present in 'Hópehely' and 'White Lady' effectively control virus infections. The mean proportion of plants showing virus symptoms was 12.8% for 'Hópehely', 16.7% for 'White Lady', and >56% for check cv. 'Desirée', which is prone to virus diseases. For the other new varieties, symptoms of PVY and PLRV infection were recorded separately (Table 4). It can be seen that in case of PVY, the *Ry_{st}* gene, responsible for extreme resistance (immunity) in Hungarian varieties, completely prevented the infection of plants and appearance of symptoms. With respect to PLRV, the varieties bred at Keszthely showed much lower infection than the checks. Re-plantations of 'Cleopatra', 'Desirée', 'Lady Rosetta' and 'Somogyi sárga kifli' resulted in a mean PVY infection of 64.9%, 47.9%,

88.8% and 91.7%, respectively, and a mean PLRV infection of 18.2%, 27.1%, 1.0% and 18.8%, respectively.

During the registration trials 1993-1996, 'White Lady' showed excellent resistance to late blight (Table 3). The horizontal resistance has been confirmed later in a field provocation trial (2000-2002) in western Hungary. The most resistant cv. 'White Lady' showed AUDPC values of 258, compared to 1427 for the most susceptible cv. 'Adora' (Gergely 2004). For 'Sárpo Mira', another Hungarian variety, Rietman *et al.* (2012) proved that its high and durable late blight resistance is based on the combination of four qualitative R genes (*R3a*, *R3b*, *R4*, *Rpi-Smira1*) and a quantitative R gene (*Rpi-Smira2*).

Common scab caused by *Streptomyces scabies* is a soil-borne tuber disease occurring every year at different rate in Hungary. Common scab causes no quantitative yield losses but a severe infection can significantly reduce the marketability. Comparing the infection indices, cvs. 'Cleopatra', 'Desirée', 'Kondor' and 'Agria' proved to be susceptible in Hungarian VCU trials over a long time period (1979-2000). Contrary, most domestic cultivars including 'White Lady' and 'Hópehely' retained their high-level field resistance (Gergely *et al.* 2003). Hungary's climatic conditions with long hot and dry periods during the growing season are favourable for scab development. Selection under these conditions, therefore, are more efficient in the identification of resistant clones which is demonstrated by the fact that seven out of eight new Hungarian

Table 3: Disease resistance of cvs. 'Hópehely' and 'White Lady' versus check cv. 'Desirée' in VCU and post-registration trials (in brackets). Means of elite and 1st generation plants of VCU trials (1993-96) and elite plots of post-registration trials (1995-98); late blight: artificial inoculation with a complex domestic race; common scab and tuber rots scored on 2 × 100 tubers after a 2 month storage.

Year	Virus diseases (% PLRV+PVY infected plants)			Late blight (% infected leaf area)		
	Hópehely	White Lady	Desirée	Hópehely	White Lady	Desirée
1993	29.8	29.8	84.3	47.6	5.0	47.6
1994	13.2	32.1	78.4	20.0	0.0	32.6
1995	16.2	17.2	85.9 (81.8)	42.6	0.0	42.6
1996	17.3	19.7	69.2 (71.9)	47.6	0.0	45.6
1997	0.7	1.4	18.2	-	-	-
1998	0.3	0.2	3.2	-	-	-
Mean	12.8	16.7	56.5 / 56.3	39.5	1.3	42.1

Year	Common scab (% index of infection)			Bacterial and fungal tuber rots (% index of infection)		
	Hópehely	White Lady	Desirée	Hópehely	White Lady	Desirée
1993	1.6	2.1	15.1	0.2	2.4	1.4
1994	2.6	1.3	16.9	0.5	1.7	1.8
1995	2.4	1.5	14.4 (6.5)	2.1	1.5	3.1 (1.5)
1996	1.4	1.4	10.4 (10.9)	4.7	0.3	5.4 (18.5)
1997	3.0	1.6	14.3	0.5	0.3	0.3
1998	1.7	1.3	11.1	2.3	0.0	2.4
Mean	2.1	1.5	13.7 / 12.5	1.7	1.0	2.4

Table 4: Disease resistance of new and check potato varieties in two years of Hungarian VCU trials (E = elite seed; e = 1st generation seed).

Cultivar	Seed	PLRV (% infected plants)		PVY (% infected plants)		Late blight (% infected leaf area)		Common scab (% index of infection)		Tuber rots (% index of infection)	
		1	2	1	2	1	2	1	2	1	2
Balatoni rózsza	E	0.2	0.0	0.0	0.0	-	-	3.4	4.7	0.3	0.4
	e	-	1.8	-	0.0	70.0	77.5	-	3.5	-	0.2
Cleopatra	E	0.1	0.0	2.9	1.7	-	-	7.9	10.2	0.8	0.8
	e	-	23.7	-	48.8	75.0	75.0	-	11.4	-	0.8
Botond	E	0.0	0.2	0.0	0.0	-	-	2.0	2.4	0.3	0.1
	e	-	1.7	-	0.0	15.0	5.0	-	2.6	-	0.3
Cleopatra	E	0.0	0.1	2.1	3.4	-	-	6.8	16.3	0.9	0.5
	e	15.5	15.6	78.7	67.1	72.5	65.0	7.8	14.1	1.4	0.7
Démon	E	0.0	0.0	0.0	0.0	-	-	4.8	5.9	0.9	0.6
	e	-	0.0	-	0.0	72.5	70.0	-	1.7	-	0.3
Desirée	E	1.6	2.8	33.5	14.6	-	-	15.1	18.3	1.7	0.7
	e	-	16.5	-	49.1	50.0	57.5	-	16.3	-	0.4
Katica	E	0.0	0.0	0.0	0.0	-	-	3.0	4.8	0.3	2.0
	e	-	0.7	-	0.0	47.5	75.0	-	3.5	-	5.8
Desirée	E	1.6	1.8	2.2	3.6	-	-	12.2	8.7	0.6	1.4
	e	29.4	35.4	50.2	44.5	57.5	56.0	11.5	10.8	0.7	3.0
Arany Chipke	E	0.1	0.0	0.0	0.0	-	-	8.1	5.8	0.8	2.0
	e	-	0.8	-	0.0	-	55.0	-	3.0	-	1.6
Lady Rosetta	E	0.3	0.1	0.0	1.3	-	-	8.2	3.5	0.9	1.8
	e	-	1.0	-	88.8	-	60.0	-	5.8	-	1.5
Basa	E	0.1	0.0	0.0	0.0	-	-	2.4	7.8	0.4	0.2
	e	-	0.9	-	0.3	0.0	0.0	-	8.0	-	0.4
Somogyi sárga kifli	E	0.6	2.9	32.4	50.7	-	-	4.5	14.5	0.9	0.3
	e	-	18.8	-	91.7	95.0	30.0	-	19.4	-	0.5

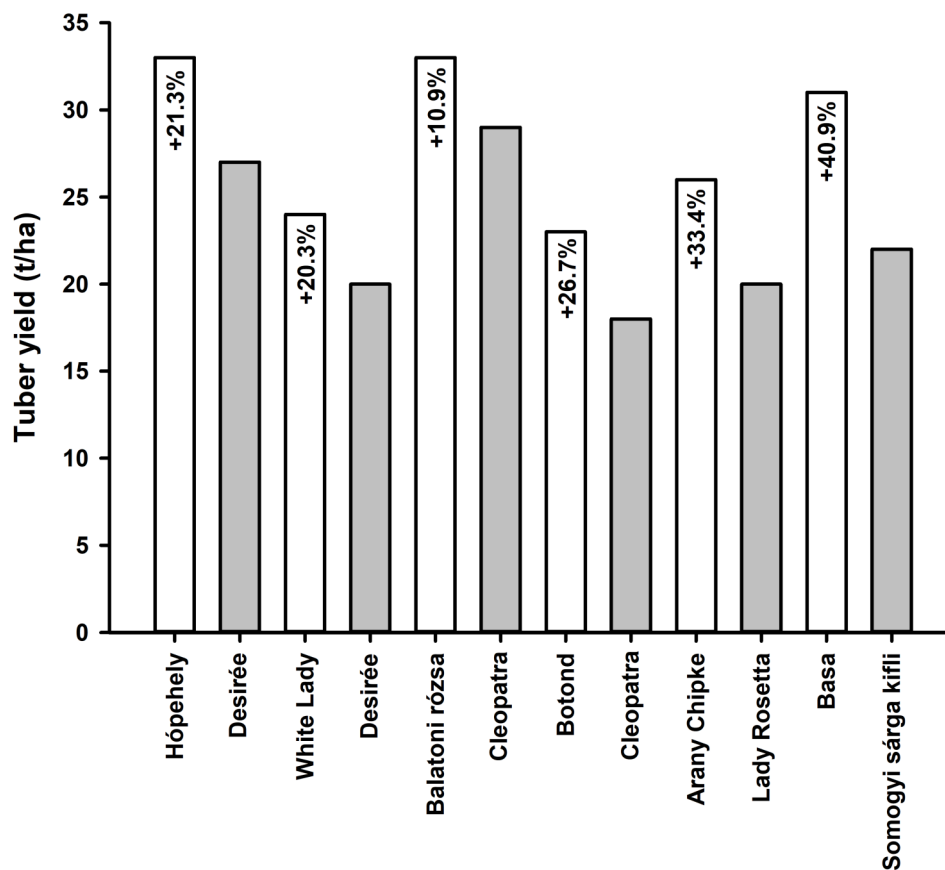


Figure 2: Pairwise comparison of the average marketable tuber yield of new Hungarian varieties (white bars) vs. their respective tested varieties in the particular second year of registration (VCU) trials planted with class A seed.

varieties showed significantly less infection than the check varieties (Table 4). The lowest infection was recorded for 'White Lady' (1.5% average of six years), while the highest rate of infection was observed for 'Somogyi sárga kifli' (19.4% in 2016) and 'Desirée' (18.3% in 2008).

In case of total tuber rots caused by fungal or bacterial pathogens, the recorded infection rates were generally low. Despite the low level of infections the new varieties showed in most cases a lower infection than the checks varieties.

Marketable yield

Marketable yield is probably the most important criteria for the acceptance of new varieties. It is also important that high-quality seed potatoes can be produced locally. This requires multiple replanting under field conditions without significant viral infestation, and loss in quantity and quality. Therefore, we focused on the comparison of the productivity of seed potatoes replanted from the first experimental year. The results are summarized in Figure 2. It is obvious that in each case the marketable yield of the new varieties outperformed the respective check variety. The highest marketable yields were recorded for 'Basa', 'Arany Chipke', 'Botond' and 'Hópehely' with +41%, +34%, +27% and +21%, respectively. Yield stability of 'Hópehely' was also confirmed by Liovic *et al.* (2008) who identified this variety as the one with both the highest yield potential and stability under their test conditions. These outstanding results are most probably the result of the

complex resistance of the new varieties. The correlation is particularly striking when the PVY infection of the 1st generation ("e") seed is related to the tuber yield.

Conclusion

The new varieties released by the potato breeding program in Keszthely have multiple resistance genes to major biotic and abiotic stresses, an important factor to avoid degeneration due to *e.g.* virus diseases under Central European conditions. Therefore, a local seed production with less input concerning plant protection can be guaranteed. The cultivation of these varieties with multiple resistance is the most economic way for farmers, increasing their market competitiveness, especially in case that the growers produce under the rules of integrated pest management or organic production (Molnár & Gergely 2019).

References

- Bradshaw JE (2016) Climate change and resistance to pests and diseases. In: Bradshaw JE (ed), Potato breeding: Past, present and future, pp 591-626. Springer Int Publ, Switzerland. DOI: 10.1007/978-3-319-23285-0_18
- EU Directive 2009/128/EC on the sustainable use of pesticides. Official J. of the EU, 24.11.2009, L 309/85.
- Gergely L, Lönhárd M, Proksza P (2003) Durability of dual resistance of potato varieties to late blight [*Phytophthora infestans* (Mont.) de Bary] and common scab [*Streptomyces scabies* (Thaxt.) Waksman et Henrici]. Acta Phytopath Entomol Hung 38: 1-6. DOI: 10.1556/aphyt.38.2003.1-2.1

- Gergely, L. (2004) Testing for resistance of potato varieties to late blight agent [*Phytophthora infestans* (Mont.) de Bary] and the effect of certain environmental factors on disease resistance. PhD dissertation, University of Veszprém.
- Leppik EE (1970) Gene centers of plants as sources of disease resistance. *Ann Rev Phytopathol* 8: 323-344. DOI: 10.1146/annurev.py.08.090170.001543
- Levy D, Veilleux RE (2007) Adaptation of potato to high temperatures and salinity - a review. *Am J Potato Res* 84: 437-456. DOI: 10.1007/BF02987885
- Liovic I, Josipovic M, Simic D, Krizmanic G, Mijic A (2008) Marketable tuber yield stability in potato. *Cereal Res Commun* 36: 87-90.
- Lönhárd M, Proksza P, Gergely L (2002) A burgonya vírusokkal szembeni ellenálló képességének állandósága és hatékonysága az országos fajtakísérletek és a hazai nemesítési tapasztalatok tükrében (Durability and efficacy of resistance of potato (*Solanum tuberosum* L.) to viruses as reflected in Hungarian variety trials and breeding). *Növénytermelés* 3: 269-280.
- Molnár J, Gergely L (2019) Improving plant immunity to diseases as an important part of integrated plant protection in the National Action Plan for Plant Protection (objectives – necessary measures – indicators). Short overview. *Agrarian Science* 2: 14-18. (In Russian)
- Polgár Z, Wolf I, Vaszili Z, Tömösközi-Farkas R, Gergely L (2010) Newest results of complex resistance breeding programme to biotic and abiotic stresses of potato. *Potato Res* 53: 396-397. DOI 10.1007/s11540-010-9180-z
- Polgar Z, Cernák I, Wolf I (2016) Potato breeding, meeting the challenges of climate change. *Lucrări Științifice, Seria Agronomie* 59: 223-227.
- Rietman H, Bijsterbosch G, Cano LM, Lee HR, Vossen JH, *et al.* (2012) Qualitative and quantitative late blight resistance in the potato cultivar Sarpo Mira is determined by the perception of five distinct RXLR effectors. *Mol Plant-Microbe Interact* 25: 910-919. DOI: 10.1094/MPMI-01-12-0010-R
- Ross H (1986) Potato breeding – Problems and perspectives. *Fortschritte der Pflanzenzüchtung* 13. Verlag Paul Parey, Berlin & Hamburg.
- Sárvári I (1967) A burgonya-leromlással szembeni rezisztenciára nemesítés egyes kérdései és eredményei (Some problems and results of breeding for resistance to potato degeneration). PhD dissertation, Keszthely, Hungary.
- van Loon CD (1981) The effect of water stress on potato growth, development and yield. *Am Potato J* 58: 51-69. DOI: 10.1007/BF02855380
- Vayda ME (1994) Environmental stress and its impact on potato yield. In: Bradshaw JE, Mackay GR (ed), *Potato genetics*, pp 239-261. CABI, Wallingford, UK.

Climate fit varieties to increase yield stability in Austria: the project KLIMAFIT

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Climate change constitutes a considerable challenge for future crop cultivation in Austria where most of the arable lands are located in regions that already suffer from water shortages and high temperatures during summer. Future climate scenarios predict long periods of drought, especially in the summer months relevant for crop production, and frequent extreme weather events in these regions until the end of the century. Future food security relies on the adoption of adaptation measures, where cropping of improved varieties plays a pivotal role. Initiated by Saatgut Austria and the Austrian Federal Ministry of Agriculture, Regions and Tourism (BMLRT), the Austrian breeders joined forces in the KLIMAFIT project coordinated by the AGES, to support plant breeding towards enhanced abiotic stress-tolerance, while ensuring yield stability. The objective of the project is to adapt agriculturally important cultivated species to climate change and to ensure a diversity of cultivated species in sustainable crop produc-

tion. In addition, genetic resources generated within the project will support sustainable long-term breeding activities in Austria to cope with climatic change while meeting the yield and quality expectations of modern varieties.

In order to develop climate fit varieties for Austria, simultaneous approaches are being pursued including the use of genomic and marker-assisted selection, as well as the phenotypic evaluation of a large number of breeding lines to evaluate their performance in different regions relevant for Austrian agriculture. In the first project year, plot trials were carried out at in total 165 locations, including 106 sites in Austria (Fig. 1). The project also included field trials at 59 locations that were carried out in other countries, e.g. in the Czech Republic, Germany, Hungary, Romania, Croatia and Poland, allowing a selection of breeding lines with broad adaptation to drought and heat stress. All sites were evaluated by the

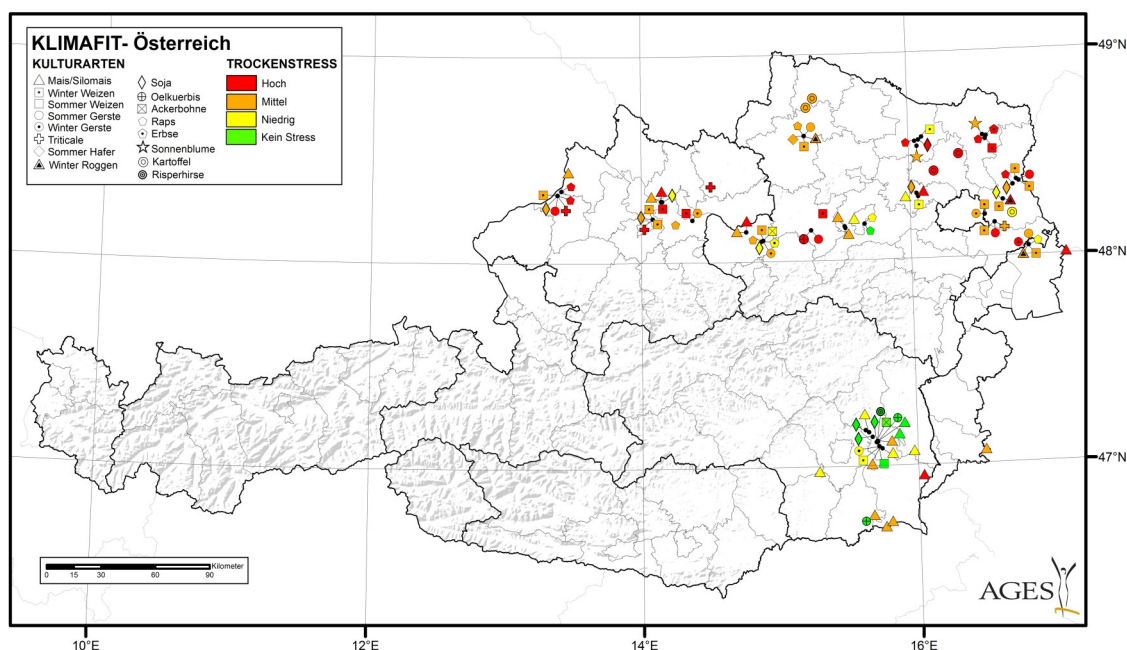


Figure 1: Overview of the Austrian KLIMAFIT test sites where field trials of the different crop species were carried out in the first project year 2018. The respective colors of the symbols show the drought stress intensity as evaluated by the breeders.

Table 1: Test sites used for breeding trials in the first project year (2018) per crop groups, classified into the four levels of drought stress intensity attributed by the breeders (1 = high, 2 = medium, 3 = low, 4 = no drought stress)

Crop group	Number of sites	Sites according to the level of drought stress (%)			
		1	2	3	4
Cereals & maize	114	29 %	46 %	19 %	5 %
Oil and protein crops	46	24 %	28 %	28 %	20 %
Potato	5	40 %	40 %	20 %	-
Total	165	28 %	41 %	22 %	9 %

breeders in terms of drought stress intensity that affected the respective crop species during the growing season. This allowed an evaluation of the extent of drought stress at each site and a comparison across locations. In a four-level ranking, the drought stress intensity was classified by the breeders by a 1 to 4 scale with 1 = high drought stress intensity, 2 = medium drought stress intensity, 3 = low drought stress intensity and 4 = no drought stress (Table 1).

The KLIMAFIT project deals with a broad spectrum of crops, including major and minor crops, to ensure varietal development in many species and, thereby, support local agricultural specialization for the future. Three main crop groups are investigated, *i.e.* cereals & maize (winter and spring wheat, winter and spring barley, triticale, rye, oats, millet and maize), oil & protein crops (rapeseed, sunflower, faba bean, field pea, soybean and oilseed pumpkin) and potato.

For statistical analysis, trials which were scored as high or medium in terms of drought stress intensity were considered as trials under abiotic stress. In order to compare the yield performance of breeding lines, check varieties were defined within each crop species for comparisons between individual trials. In general, two analyses were used to evaluate the effect of drought stress on the yield performance of the tested breeding lines. In the first step, the yield performance under drought stress was analysed. In the second step, the yield performances of all lines was analysed

across all experimental conditions, independent of the defined drought stress intensity. The adjusted means of the breeding lines were compared with the adjusted means of the check varieties and the breeding lines with stable and high yield performances were identified.

In the scope of the KLIMAFIT project, several breeding lines have already been submitted to the official VCU test in Austria. We expect the release of new varieties with improved abiotic stress tolerance and enhanced performance despite adverse climatic conditions in the forthcoming years.

Keywords

Abiotic stress tolerance · breeding · climate change · drought · global warming · heat

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Assessment of baking quality of wheat in the value chain

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Abstract

The reduction of nitrogen fertilization and in consequence the decrease of protein content affects the baking quality of wheat. This was shown in a multi-environment trial with sixteen varieties grown under three different fertilizer levels where baking volume and rheological properties indicated a minor quality of wheat at lower fertilizer levels. However, the results suggested that the genetic effect of the variety possibly related to an improved protein quality could compensate lower protein contents.

Keywords

Baking quality · grain yield · nitrogen fertilisation · protein content
· *Triticum aestivum*

Introduction

Bread wheat (*Triticum aestivum*) is the most important food crop in the world. Roughly, one third of the wheat produced in Germany is used for human nutrition, a sixth goes to other EU countries and another sixth to the international markets. Most of the production is used for feeding and industrial purposes (BLE 2019). Baking quality is a central surplus for trading the commodity wheat. An acceptable specific weight and high falling number are basic requirements. The measurement of baking quality is complex and cannot be performed by the elevators and traders. In contrast, protein content can be easily determined by NIR or NIT spectroscopy. Therefore, it is the main criteria for a price surcharge. Millers conduct additional analyses such as sedimentation volume and content and quality of gluten. A common frame exists for the classification of wheat, but it is not fixed in the market and depends on the regional supply and demand. Milling wheat for bread, A and E quality wheat are the main categories in Germany. German wheat for international export at the seaport is competitive at a protein level of 12.5 %.

Baking quality of wheat varieties is determined by the Bundesortenamt and documented in the *Beschreibende Sortenliste*. Here, besides milling and baking quality parameters, especially the baking volume of rolls is an important criterion. Protein content as classification criterion was abandoned in 2019, but is still reported and recognized as an important trait. This opened the opportunity

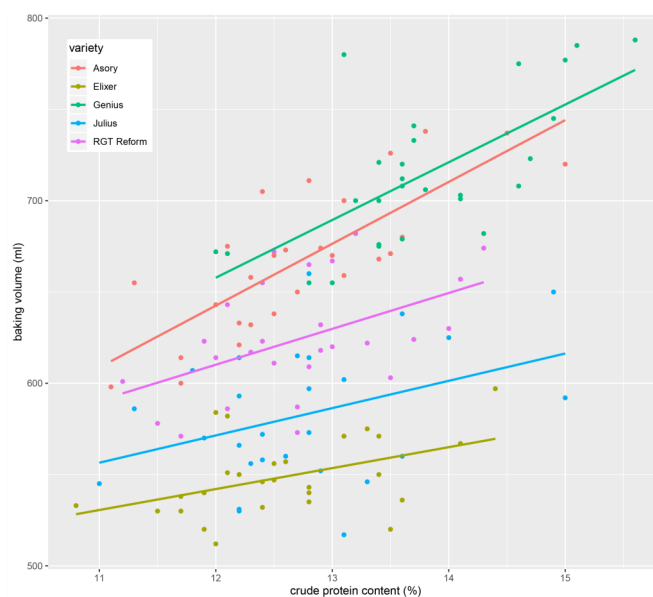


Figure 1: Baking volume in relation to crude protein content in German VCU trials of winter wheat (S1, S2, S3 2015-2017)

for high-yielding varieties with good baking quality to enter the higher quality classes.

Protein content has a significant influence on baking quality, but the major factors are the production environment and the genetics of the variety. At a similar protein level, varieties can differ considerably in baking volume. Considering the relevant protein content range of A quality wheat (12.5%-13.5%), the varieties were clearly separated with respect to baking volume (Figure 1). Within this range, the influence of protein content on the complex trait baking volume is clearly visible but the major determinant is the genetically fixed protein composition.

Regulation on nitrogen fertilisation

In Austria the reduction of nitrogen (N) application started several years ago. In Germany, the limitation of N fertilization is a major topic now (BMJV 2017, LfL 2020). Following the new regulation, N application in wheat often has to be reduced by about 10-20%. Red zones are mapped as regions with problematic nitrate concentrations in the groundwater. In red zones additional regula-

tions will be fixed most likely next year, such as further reduction N application by 20%.

For the most important groups, the A/B and E quality wheats, the N demand is fixed at 230 kg N ha⁻¹ and 260 kg N ha⁻¹, respectively, at a yield level of 80 dt ha⁻¹. Mineralised N in the soil has to be measured and subtracted from the demanded N nutrition. An average of 60 kg mineralised N ha⁻¹ were observed in Bavarian soils in early spring. This resulted in a moderate N fertilization of 170 kg N ha⁻¹ for A quality wheats. The questions arise whether the farmers are able to fulfil the demand of the regional millers and what strategies are useful for the different markets.

Material and Methods

For estimating baking quality at different protein levels a series of production trials were started with three N fertilizer levels in 2015/2016 at four locations; 2018 was excluded from analysis due to drought during grain filling and thus a narrow differentiation in protein content. The set of 16 varieties comprised relevant E and

A quality varieties and historical high-baking quality varieties. After the second year, the set was adapted with actually relevant varieties. Three fertilizer levels were applied. Depending on yield expectation and the actual conditions of vegetation at each location the levels of N-fertilization differed by 20-40 kg N ha⁻¹. The amount of N followed the current fertilizer regulation for A/B and E quality wheats and the former N level (+30 kg N ha⁻¹) in 2015/16 and 2016/17. In 2017/18 the locations were changed, the number of locations reduced to three and the set of varieties was updated. Due to the discussion on 'red zones' a new fertilizer level with reduced N was introduced and the "former N level" was excluded. Crude protein content was analysed with near-infrared spectroscopy. The baking tests were conducted following the RMT baking test for rolls (Anonymous 2016) based on flour standardised to 550 mg kg⁻¹ ash. Water absorption and dough stability were determined with the Farinograph (Brabender, Duisburg) with a kneading unit for 50 g flour (ICC 115/1) and extensibility with the Extensograph (Brabender, Duisburg) using a resting time of 45 min (ICC 114/1). Analysed data of all years are available at <https://www.lfl.bayern.de/ipz/getreide/> or upon request.

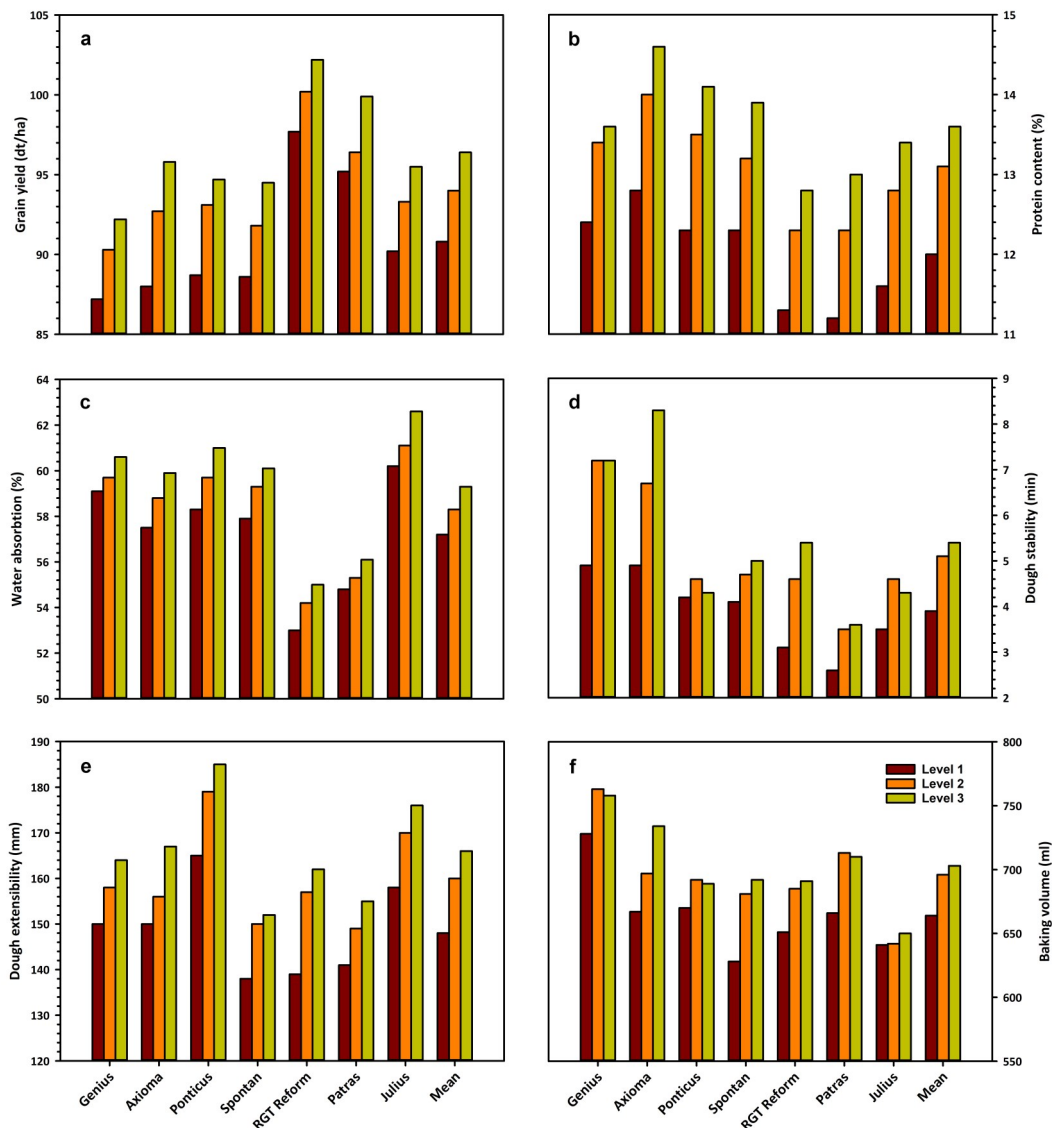


Figure 2: Trial data of selected varieties conducted at three N fertilizer levels with consequences on (a) grain yield, (b) protein content, (c) water absorption, (d) dough stability, (e) dough extensibility, and (f) baking volume.

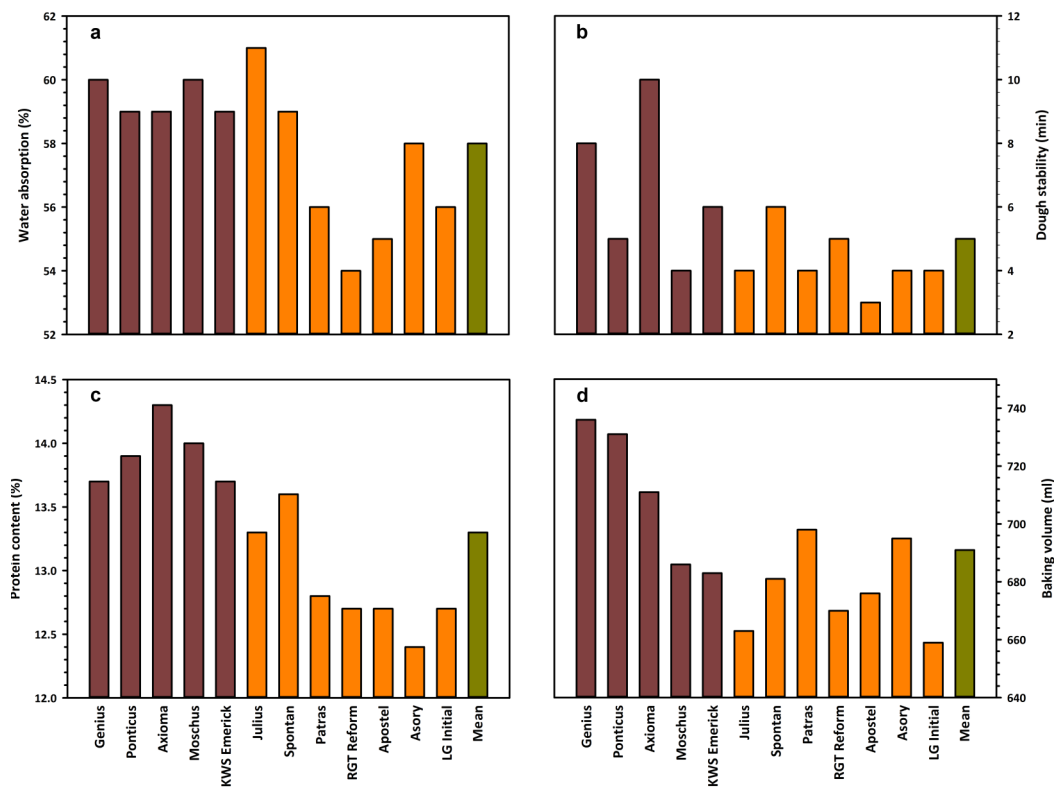


Figure 3: Data of the trial averaged over the three fertilizer levels containing the following parameters: (a) water absorption, (b) dough stability, (c) protein content, (d) baking volume. Quality classes are indicated by different colors (E: Genius to KWS Emerick; A: Julius to LG Initial).

Results and Discussion

Enhanced fertilisation by 30 kg N ha^{-1} increased yield by 3 and 2 dt ha^{-1} and crude protein content by 1% and 0.5%, respectively (Figures 2a,b). The usual protein levels of 13% and 14% for trading of A and E quality varieties were only reached at the highest fertilizer level which does not fit to the limits of the regulation for many practical situations. All baking quality parameters were clearly affected by the increase of protein content. Each cultivar achieved a higher baking volume at fertilizer level 2 (Figure 2f). Up to level 3 the improvement in protein content and baking volume was less. The average baking volume increased by about 30 ml per one percent absolute increase of protein content which fitted very well the often observed relation.

The gain of the specific varieties was dependent on the elasticity of the dough. The variety 'Axioma' which is characterized by very resistant dough probably profited by the disproportionally enhanced gliadin content which makes the dough more extensible. The minor reaction of varieties with a more elastic dough implicates that enhanced protein content does not directly enhance baking volume but it cannot be ruled out that it is useful for the blending of wheat lots. The variety 'Patras' outperforms all other A quality varieties.

In relation to the low protein content of 11% to 13% the baking volume reaches up to $700 \text{ ml } 100 \text{ g}^{-1}$ flour. In the milling industry the variety 'Patras' is well known as a variety with good and uncomplicated baking characteristics even with slightly lower, *i.e.* 12.5%, protein content. For all of the varieties, increasing protein content affects the complete cluster of quality traits. The effects of

increase of N fertilisation on yield and protein content, and as consequence on water absorption, dough stability and extensibility are shown in Figure 2.

In 2017/2018 a few newly registered A quality varieties with generally low protein content were integrated in the trial. Based on the registration trials it was known that 'Apostel' and 'Asory' combined low protein content and a relatively high baking volume. 'Asory' reached the same level of baking volume as the E quality varieties 'Moschus' and 'KWS Emerick' at a 1.0-1.5% lower protein content. Water absorption and dough stability were at a comparable level to other A quality varieties with normal protein content (Figure 3).

Conclusion

Under the new strict fertilizer regulation it will be difficult for the farmers to produce enough wheat with a high protein content to which the millers and the baking industry were used. Different adaptation strategies could be developed depending on the market setting. Based on quality aspect, the wheat market could be split in three categories:

- 1 High quality varieties, *e.g.* E-quality varieties which are stored separately.
- 2 Protein content is the main quality criterion. Wheat lots are separated based only on their protein content. Blending of lots will be accepted irrespective of the quality group of the varieties.
- 3 The most sustainable and efficient class encompasses wheats which are separated based on both protein content and quali-

ty group. Separation can be further improved taking into account specific quality traits of the varieties (*e.g.* dough characteristics). This will allow lowering the protein content while preserving the robustness of the processing in the bakery.

At the moment, the market does not offer a reward to quality-improved lots. Because of the fear that high quality wheat could increase in price and be limited on the market, the milling industry thinks extensively about future strategies. They are quite interested in specific quality attributes of the varieties. But it remains to be seen if they will honour high quality varieties at a lower protein level.

Acknowledgments

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References

Anonymous (2016) Standard-Methoden für Getreide, Mehl und Brot mit allen aktuellen ICC-Standards. Hrg. Arbeitsgemeinschaft Getreideforschung e. V. (AGF), Detmold.

BLE (2019) Bericht zur Markt- und Versorgungslage, Getreide 2019. Bundesanstalt für Landwirtschaft und Ernährung (BLE), Bonn.

BMJV (2017) Verordnung über die Anwendung von Düngemitteln, Bodenhilfsstoffen, Kultursubstraten und Pflanzenhilfsmitteln nach den Grundsätzen der guten fachlichen Praxis beim Düngen (Düngeverordnung - DüV). Bundesministerium der Justiz und für Verbraucherschutz (BMJV), Berlin. [https://www.gesetze-im-internet.de/d_v_2017/D%C3%BCV.pdf]

LfL (2020) Düngeverordnung. Bayerische Landesanstalt für Landwirtschaft (LfL), München. [<https://www.lfl.bayern.de/iab/duengung/032364/index.php>; accessed March 3, 2020]

Loaf volume - Is it predictable? An improved method to predict loaf volume in winter wheat by regression models

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Crude protein content (PC) and Zeleny-sedimentation value (SV) are important indirect traits to evaluate baking quality of wheat. Loaf volume (LV), determined by the Rapid-Mix-Test (RMT), is used to describe the baking ability of wheat varieties. However, the routine application of such baking tests is time consuming and laborious. Therefore, in German official variety trials (VCU), LV is calculated by the Bolling formula based on the indirect traits PC and SV in the framework of the special harvest and quality assessment (*Besondere Ernte- und Qualitätsermittlung (BEE) des BMEL*). The formula was introduced by Bolling in 1969. Two regression equations are used: (a) $LV = 410 + 10 \times PC + 3 \times SV$ for varieties of the E and A quality groups, and $LV = 306 + 17 \times PC + 3 \times SV$ for B-varieties (Bolling 1969). It could be shown that during the last 50 years breeding resulted in a change of the protein quality of varieties in a way that the prediction accuracy of the Bolling formulas decreased continuously. In a comparison of the LV predicted for BEE data by the Bolling formulas with LV assessed by the RMT

from VCU trials, we found that the predicted LV of varieties was considerably overestimated as compared to the realized LV in VCU trials (Laidig et al. 2017).

The analysis to find an improved method for the prediction of LV was based on two data sets (VCU trials and mill samples) including only registered varieties of quality groups E, A and B. Available were the RMT-LV, PC and SV in both data sets covering the years 1987-2016. The VCU data set ($n = 9253$) provides a solid basis for a robust prediction model comprising a broad spectrum of varieties and environmental conditions congruent to the BEE samples. The mill samples ($n = 3931$) arise from on-farm grain lots provided by mills. In a first step, we applied to both data sets a simple linear regression model with LV as dependant, and PC and SV as independent variables for each year and quality group separately. Then we plotted the estimated regression coefficients against the years. The plots indicated negative time trends for the intercept

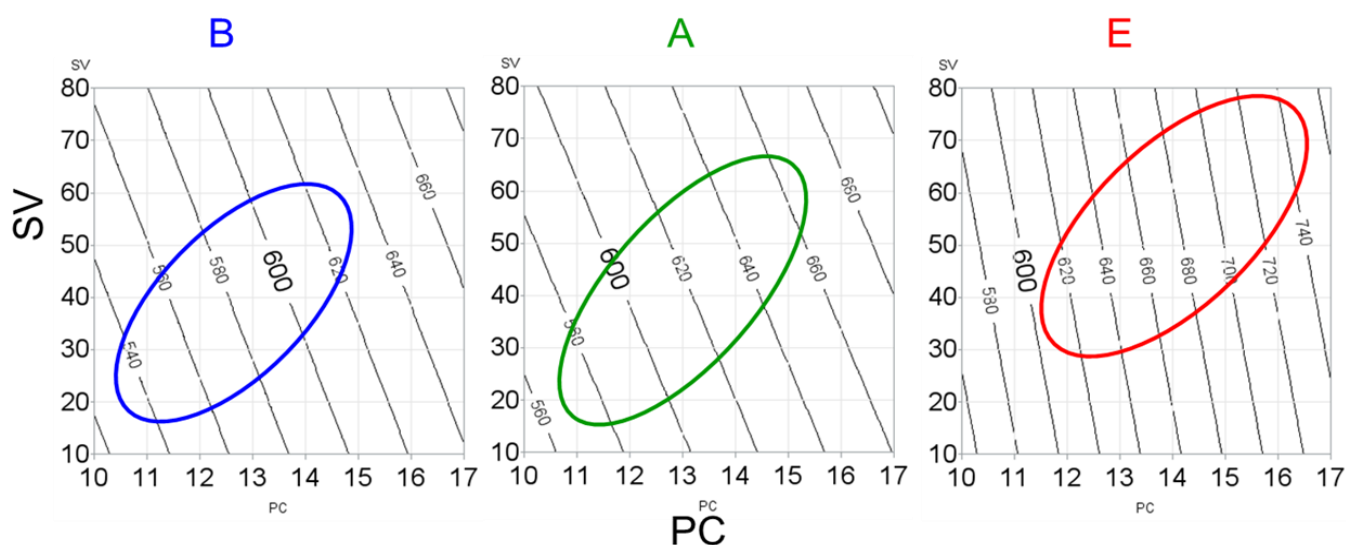


Figure 1: Contour plots predicting loaf volume (LV, mL) for year 2018 with regression equation Opt2q estimated from VCU data 1998-2017 for varieties of B, A and E quality. A contour line indicates the combination of protein content (PC) and Zeleny sedimentation value (SV) predicting the same LV. The confidence ellipses include about 95% of the samples.

and the regression coefficient for SV, but a positive trend for PC. The plots further showed a considerable deviation of the year-wise coefficients from the corresponding coefficients of the Bolling formulas. We considered the time trends when generating regression models to be tested. The predictive power of four model options was compared with the Bolling formulas (Opt0q).

For option 1 (Optx1) a single regression was calculated not separately for quality groups and no time trends were taken into account. For option 2 (Opt2x) regression was not calculated separately, but time trend was taken into account. For option 3 (Opt1q) regression was calculated separately for quality groups, but time trend was not taken into account. Finally, for option 4 (Opt2q) regression was calculated separately and time trend was taken into account.

The regression equations obtained by the four model options and given by Bolling's formulas were subject to four independent evaluation schemes. For two direct prediction schemes, samples from VCU/mills were predicted by equations derived from VCU/mill data set, and for two cross prediction schemes, samples mill/VCU were predicted by equations derived from VCU/mill data set. For each evaluation scheme 10 cycles were carried out by calculating the regression equations from data including 20 years to predict LV for the 21st year. For the 10th cycle, for example, the regression equations were estimated from years 1996-2015 to predict LV for samples from year 2016. The prediction accuracy was measured by the root of the mean squared deviations of the observed vs. the predicted LV (sMSD). The average sMSD across the four evaluation schemes was 76.0 for the Opt0q (Bolling formulas), 46.7 for Opt1x, 43.5 for Opt2x, 44.5 for Opt1q and 40.3 for Opt2q. The result indicates that Opt2q predicts LV with the highest accuracy, hence, it is best to take the time trend in the regression coefficients into account and predict the samples separately for each quality group. The results reported by Laidig et al. (2018) further showed that Opt2q provided the most robust predictions across quality groups. When the regression estimates from Opt2q of the most recent 20 years of VCU data are used, then two to three new years of BEE samples can be predicted.

In Figure 1, contour plots are shown for predicting LV for year 2018 with regression equation Opt2q estimated from VCU data 1998-2017. A contour line indicates the combination of PC (x-axis) and SV (y-axis) which predict the same LV. Within the confidence ellipses, about 95% of the samples are located. The three plots reveal that LV is increasing and that the contour lines are getting steeper from B- to E-varieties. For E-varieties, LV is mainly determined by PC, whereas for B-varieties both PC and SV are determining LV.

This study has shown that the model Opt2q improves the prediction accuracy of LV considerably, and that the procedure can be updated by using VCU results and can easily be applied. In the future, this procedure will be applied to predict BEE samples and it can also be used by the complete processing chain for baking wheat.

Keywords

Baking quality · protein content · regression analysis · *Triticum aestivum* · Zeleny sedimentation

References

- Bolling H (1969) Wissenschaftliche Grundlage für eine Gradierung von Weizen. Mühle+Mischfüttertechnik 106: 799-801.
- Laidig F, Piepho HP, Rentel D, Drobek T, Meyer U, Huesken A (2017) Breeding progress, environmental variation and correlation of winter wheat yield and quality traits in German official variety trials and on-farm during 1983-2014. Theor Appl Genet 130: 223-245. DOI: 10.1007/s00122-016-2810-3
- Laidig F, Piepho HP, Hüsken A, Begemann J, Rentel D, et al. (2018) Predicting loaf volume for winter wheat by linear regression models based on protein concentration and sedimentation value using samples from VCU trials and mills. J Cereal Sci 84: 132-141. DOI: 10.1016/j.jcs.2018.07.015

Genetic analysis of baking quality using a multiparental winter wheat population

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The improvement and maintenance of baking quality is a key target in winter wheat breeding. Phenotypic selection for high quality is time-consuming and labour-intensive and is therefore only feasible in advanced generations. Although genetic loci encoding for storage proteins and puroindolines have been shown to play an important role, the knowledge about the genetics of wheat quality is still limited. To gain further insights, we genetically analysed the eight-way multiparent advanced generation intercross population BMWpop for important quality parameters and agronomic traits.

The BMWpop comprises 394 recombinant inbred lines and was shown to represent a major part of the allelic diversity of German winter wheat. The population was evaluated in field trials with two replications in eight environments at six German locations over a period of four years. Phenotypic data were collected for quality traits, such as loaf volume (LV), grain protein content (GPC), grain hardness (GH) and wet gluten content (WGC). To study the relationship between wheat quality and agronomic traits, we also recorded data for heading date (HD), plant height (PH), thousand grain weight (TGW) and grain yield (GY). Grain protein deviation (GPD) was derived from a linear regression of GPC on GY. The BMWpop was genotyped using an Illumina® Infinium® 15+5k single nucleotide polymorphism (SNP) array and diagnostic markers for the *Glu-1* and *Pinb-D1* loci. The genetic map comprising 5436 SNP markers was available from a preceding study. Quantitative trait loci (QTL) were detected by simple interval mapping using the R package mpMap.

As expected from previous studies, LV was positively correlated with GPC ($r = 0.35$) and negatively correlated with GY ($r = -0.26$). This negative correlation is probably the result of the inverse relationship between GY and GPC ($r = -0.55$). Surprisingly, we also observed a negative correlation between LV and WGC ($r = -0.12$), indicating that WGC is not a reliable predictor for LV. Interval mapping for LV revealed that *Glu-D1* was consistently detected, whereas other QTL on chromosomes 3A, 3B and 6D were only found in single environments. *Glu-D1* explained a major part of the phenotypic variance ($R^2 = 0.1$) and showed an additive effect of $\alpha_{max} = 58$ ml. For GPC, 11 QTL were identified with a maximum explained phenotypic variance of $R_{max}^2 = 0.11$ and a maximum additive effect of $\alpha_{max} = 0.8\%$. Nine QTL controlling GH were detected ($R_{max}^2 = 0.10$; $\alpha_{max} = 4\%$), among which *Pinb-D1* showed the most significant effect. *Glu-D1* has been identified as the major locus controlling WGC together with two minor QTL on chromosomes 2B and 6B ($R_{max}^2 = 0.13$; $\alpha_{max} = 4.1\%$). Subsequent analysis of wheat quality using diagnostic markers for *Pinb-D1* confirmed the

assumption that the wild-type *Pinb-D1a* allele, which is associated with soft grains, leads to lower LVs compared to the mutated *Pinb-D1* variants. Targeted analysis using diagnostic markers for *Glu-1* loci showed that, contrary to our expectations, *Glu-1* alleles exhibited opposite effects for LV and WGC. The alleles *Glu-A1a*, *Glu-B1a* and *Glu-D1d* increased LV but decreased WGC. This suggests that *Glu-1* loci are causal for the negative correlation between LV and WGC. The observation that the null allele *Glu-A1c* yielded a higher WGC compared to *Glu-A1a* and *Glu-A1b* indicates that the effects of *Glu-1* may be explained by functional properties. Interval mapping of GY and GPD resulted in ten ($R_{max}^2 = 0.10$; $\alpha_{max} = 7.8$ dt ha⁻¹) and eight ($R_{max}^2 = 0.10$; $\alpha_{max} = 0.9\%$) stable QTL, respectively. Six of these loci coincided with QTL for GPC. QTL detected for GPD showed varying trade-off effects and are interesting breeding targets to counter the inverse relationship between GY and GPC. Further research will be undertaken to deepen the understanding of these QTL and their effect on end-use quality and agronomic traits.

Keywords

Grain protein deviation · loaf volume · MAGIC · QTL · *Triticum aestivum*

Acknowledgments

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References

- Huang BE, George AW (2011) R/mpMap: a computational platform for the genetic analysis of multiparent recombinant inbred lines. *Bioinformatics* 27: 727-729. DOI: 10.1093/bioinformatics/btq719
- Stadlmeier M, Hartl L, Mohler V (2018) Usefulness of a multiparent advanced generation intercross population with a greatly reduced mating design for genetic studies in winter wheat. *Front Plant Sci* 9: 1825. DOI: 10.3389/fpls.2018.01825

Genomic prediction of baking quality in winter wheat

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Many methods for phenotyping of wheat quality traits are time-consuming, expensive, and require relatively large amounts of grain. Genetic markers can be used to reduce the need for phenotyping in breeding programs, and thereby to increase the number of lines that can be assessed.

The goals of this study were to identify SNP markers associated with wheat quality traits in a commercial breeding program, to evaluate models for genomic prediction of quality traits, and to study factors that can affect the predictive accuracy of genomic prediction models. For genomic prediction, a very large number of markers are used for prediction of breeding values. Lines that have been genotyped and phenotyped are used as training set for models, which can then be used for predicting breeding values of other lines based on their genotypes.

A total of 635 F₆ winter wheat lines from two breeding cycles of the Danish plant breeding company Nordic Seed A/S were phenotyped for the nine quality traits grain protein content, thousand-kernel weight, test weight, flour yield, Zeleny sedimentation, falling number, and Alveograph parameters P (dough tenacity), L (extensibility), and W (strength). The lines were genotyped using a 15k SNP chip array resulting in 10 802 informative SNP markers. GWAS were performed by single marker regression. Genomic predictions were performed using GBLUP or Bayesian Power Lasso models. Predictive accuracies of the models were evaluated using different cross-validation strategies (leave-one-out, leave-family-out, leave-set-out and k-fold).

Each of the quality traits had a moderate to high narrow sense heritability. The GWAS indicated that the traits were controlled by many QTL with small effects. Few SNP markers with large effects on one or more of the traits were identified on chromosomes 1B, 1D, and 5D. Models for genomic prediction of the traits mostly had intermediate to high predictive accuracies. The accuracies were highest for Zeleny sedimentation and Alveograph W, and lowest for grain protein content. Bayesian Power Lasso models allow a stronger shrinkage of small marker effects and a weaker shrinkage of large marker effects compared to GBLUP models. This

might represent the genetic architecture of traits more accurately, if the traits are controlled by few QTL with large effects. However, GBLUP and Bayesian Power Lasso models resulted in very similar accuracies for the traits studied here. The predictive accuracies were affected by the heritability of the traits, genotype by environment interactions, the number of lines in the training set, and the genetic relationship between lines in training and validation sets. The results of the study might facilitate implementation of genomic prediction of quality traits in wheat breeding programs.

Keywords

Genetic markers · genomic selection · *Triticum aestivum* · wheat breeding

Acknowledgments

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References

- Kristensen PS, Jahoor A, Andersen JR, Cericola F, Orabi J, *et al.* (2018) Genome-wide association studies and comparison of models and cross-validation strategies for genomic prediction of quality traits in advanced winter wheat breeding lines. *Front Plant Sci* 9: 69. DOI: 10.3389/FPLS.2018.00069
- Kristensen PS, Jahoor A, Andersen JR, Orabi J, Janss L, Jensen J (2019) Multi-trait and trait-assisted genomic prediction of winter wheat quality traits using advanced lines from four breeding cycles. *Crop Breed Genet Genom* 1: e190010. DOI: 10.20900/cbagg20190010
- Kristensen PS, Jensen J, Andersen JR, Guzmán C, Orabi J, Jahoor A (2019) Genomic prediction and genome-wide association studies of flour yield and Alveograph quality traits using advanced winter wheat breeding material. *Genes* 10: 699. DOI: 10.3390/genes10090669

Impact of environmental conditions on the occurrence of ATIs in bread wheat

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Essential parameters in cereal production, such as protein quantity and quality, are strongly influenced by environmental conditions, especially during grain filling. Amylase-trypsin inhibitors (ATIs), a group of water-soluble wheat proteins, play an important role in the occurrence of wheat sensitivity and other wheat-related diseases. However, the development of ATIs during grain filling and maturation as well as the environmental impact on the accumulation of ATIs in the crop plant have not been studied so far. ATIs are crucial for the natural defense mechanism of the cereal plant itself as they inhibit *e.g.* insect digestive enzymes. The inhibitory potential of ATIs is known to be strongly dependent on their 3D-structure and their degree of aggregation (monomeric, dimeric or tetrameric). In this study, the synthesis of ATIs during grain development and environmental impacts on the accumulation were studied.

In order to evaluate the progress of ATI synthesis in wheat, samples were collected successively from the common wheat variety 'Arnold' at eight different growth stages after anthesis. In order to inhibit further reactions or enzymatic activity, the grains were frozen and ground under liquid nitrogen immediately after har-

vest. The whole meal samples were freeze-dried before they were analysed for their kernel size, crude protein content and amount of salt-soluble protein. Furthermore, the occurrence of ATIs in the sample was determined by MALDI-TOF MS, and their trypsin inhibitory activity (TIA) via an enzymatic assay. Based on the obtained results, analytical data for 17 winter wheat samples (*Triticum aestivum* cv. 'Arnold'), derived from nine different test sites and two growing seasons, were correlated with the respective climatic and management data, *e.g.* temperature, precipitation and fertilization, in order to evaluate the impact of climatic conditions on ATIs. MALDI-TOF MS revealed that the accumulation of ATI monomers started around two weeks after anthesis (Figure 1), whereas their enzymatic activity was not detectable before day 25 after anthesis. This time lag of over one week might be caused by a preceding assembling step of ATI monomers to dimers and tetramers, which seems to be essential for their inhibitory activity.

Characterization of the multi-environment (MET) 'Arnold' samples ($n=17$) revealed significant changes of TIA between the two growing seasons (*i.e.* 2016 and 2017), whereas ATI concentrations remained relatively stable. As a further consequence, the inhibitory

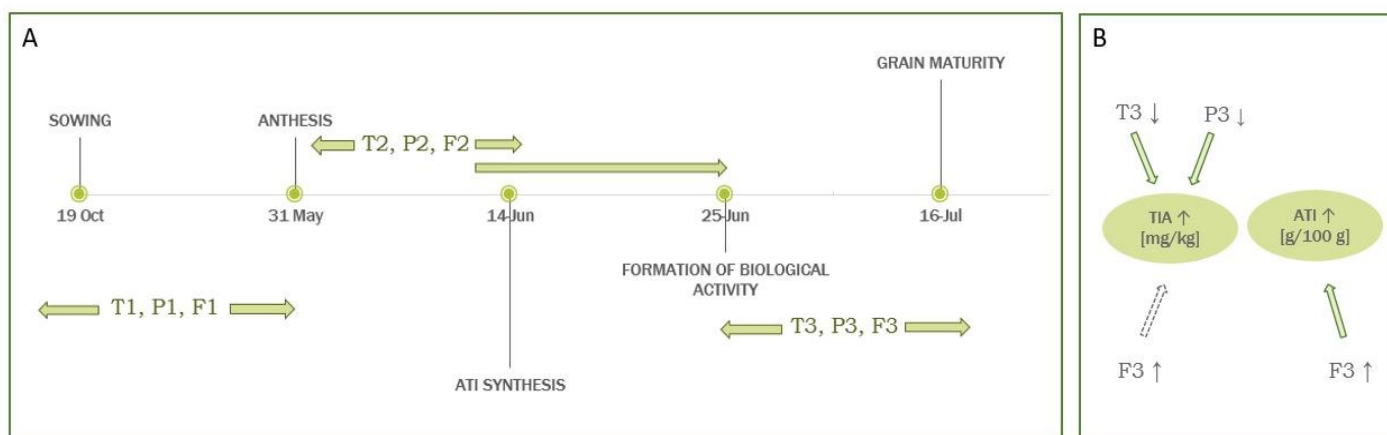


Figure 1: (A) Critical stages of grain development from sowing until final maturation of the wheat kernels (T, P and F refer to temperature, precipitation and fertilization, respectively, of the corresponding growth stage); (B) Influencing factors on trypsin inhibitory activity (TIA) and ATI concentrations (ATI) (solid lines indicate significant ($p < 0.05$), dashed lines non-significant correlations).

potential of ATIs was found to be independent of the present ATI concentrations in the wheat grain. Identification of proteins by peptide mass fingerprinting revealed that different ATIs show varying affinities towards trypsin. ATI concentrations were significantly increased in samples that were grown under conventional conditions (fertilization) compared to organic samples. However, fertilization had no significant impact on TIA values.

Based on the results derived from the harvest trial, the growing seasons of the 'Arnold' MET samples were subdivided into three development phases, from sowing to anthesis (S1), from anthesis to ATI critical date (either ATI synthesis or formation of biological activity) (S2), and from ATI critical date to grain maturity (S3). Temperature and precipitation data for every growing phase and location ($n=9$) were collected and correlated to the corresponding ATI amounts and enzymatic activities. TIA was found to be negatively affected by temperature and precipitation, while ATI concentration was influenced only by fertilization.

In conclusion, the presented results showed that ATIs were undergoing an assembling process during grain maturation before developing their enzymatic activity. Additionally, this study demonstrated that environmental factors, such as temperature and precipitation, mainly affect the enzymatic activity of ATIs in the grain samples rather than ATI contents.

Keywords

Amylase-trypsin inhibitors · biosynthesis · *Triticum aestivum* · wheat sensitivity

Acknowledgments

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References

- Call L, Reiter EV, Wenger-Oehn G, Strnad I, Grausgruber H, *et al.* (2019) Development of an enzymatic assay for the quantitative determination of trypsin inhibitory activity in wheat. *Food Chem* 299: 125038. DOI: 10.1016/j.foodchem.2019.125038
- Call L, Kapeller M, Grausgruber H, Reiter E, Schoenlechner R, D'Amico S (2020) Effects of species and breeding on wheat protein composition. *J Cereal Sci* 93: 102974. DOI: 10.1016/j.jcs.2020.102974
- Zevallos VF, Raker V, Tenzer S, Jimenez-Calvente C, Ashfaq-Khan M, *et al.* (2017) Nutritional wheat amylase-trypsin inhibitors promote intestinal inflammation via activation of myeloid cells. *Gastroenterol* 152: 1100-1113.e12. DOI: 10.1053/j.gastro.2016.12.006

Map the MAGIC - dissecting complex traits in wheat

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High anther extrusion is of advantage to promote cross pollination and to ensure high level of pollen availability for hybrid wheat seed production and is therefore of major interest in hybrid breeding programs. Hence, the genetic architecture of anther extrusion was studied in the multi-parental population MAGIC-WHEAT WM-800 by genome wide association studies (GWAS) with a fivefold cross-validation to identify genes involved in a non-cleistogamous flowering performance. Phenotypic data for 800 genotypes were collected in two years, showing strong variation for AE between the founder genotypes as well as within the WM-800 population and high heritability of 75.7%, optimal requirements for a GWAS with 27 685 SNPs, a combination of 15k Illumina array and 135k Affymetrix array.

GWAS revealed *Rht-B1* and *Rht-D1* as the major genetic players, whereas the short straw allele results in a lower anther extrusion. The insensitivity to gibberellin acid in the mutant allele lets speculate that the reduced cell division and elongation leads to shorter anther filaments.

Besides the known genes for plant height, novel genetic regions determining anther extrusion were estimated. Chromosome 1B harboured a favourable allele from 'Meister' which explained 5.3% of the phenotypic variation. According to the phenotypic data, 'Meister' is the genotype with strongest anther extrusion among all founder.

Anther extrusion can as well be influenced by swelling of the lodicule at the base of the floret. Their expansion forces the lemma and palea apart, enabling the anthers to emerge. The significant SNP on chromosome 2A is localized in a genetic region with functional annotation in wheat; a plant invertase-pectin methyltransferase inhibitor. These results were strengthened by the protein evidence of a pectin methyl esterase inhibitor of *Aegilops tauschii* at the same genetic region. Pectin methyltransferase (PME) is the first enzyme acting on pectin, a major component of plant cell wall, and decisive in plant development. Therefore, the estimated genetic region at QTL on 2A could play an important role in plant

development regarding anther extrusion. Additionally, the significant region could coincide with the genetic location of the homoeologous orthologs of the barley cleistogamy gene *Cly1*, designated as *TaAP2-A* on the long arm of chromosome 2.

Further, a GWAS for *Fusarium* susceptibility and plant height estimated favorable and unfavorable overlapping regions for the examined traits. Therefore, we postulate that the results of GWAS for anther extrusion in combination with higher *Fusarium* tolerance would improve the modern gene pool by marker assisted selection.

References

- Graham S, Browne RA (2009) Anther extrusion and *Fusarium* head blight in European wheat. *J Phytopathol* 157: 580-582. DOI: 10.1111/j.1439-0434.2008.01524.x
- He X, Singh PK, Dreisigacker S, Singh S, Lillemo M, Duveiller E (2016) Dwarfing genes *Rht-B1b* and *Rht-D1b* are associated with both Type I FHB susceptibility and low anther extrusion in two bread wheat populations. *PLoS One* 11: e0162499. DOI: 10.1371/journal.pone.0162499
- Lu Q, Lillemo M, Skinnnes H, He X, Shi J, Ji F *et al.* (2013) Anther extrusion and plant height are associated with Type I resistance to *Fusarium* head blight in bread wheat line 'Shanghai-3/Catbird'. *Theor Appl Genet* 126: 317-334. DOI: 10.1007/s00122-012-1981-9
- Sannemann W, Lisker A, Maurer A, Léon J, Kazman E *et al.* (2018) Adaptive selection of founder segments and epistatic control of plant height in the MAGIC winter wheat population WM-800. *BMC Genomics* 19: 559. DOI: 10.1186/s12864-018-4915-3
- Skinnnes H, Semagn K, Tarkegne Y, Marøy AG, Bjørnstad Å (2010) The inheritance of anther extrusion in hexaploid wheat and its relationship to *Fusarium* head blight resistance and deoxynivalenol content. *Plant Breed* 129: 149-155. DOI: 10.1111/j.1439-0523.2009.01731.x
- Skinnnes H, Tarkegne Y, Dieseth J, Bjørnstad Å (2008) Associations between anther extrusion and *Fusarium* head blight in European wheat. *Cereal Res Commun* 36: 223-231. DOI: 10.1556/CRC.36.2008.Supp.B.19

Investigating resistance to powdery mildew in winter wheat using nested associating mapping (NAM) populations

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Wheat (*Triticum aestivum* L.) is one of the world's most important staple food crops and powdery mildew (PM) is one of the most devastating fungal diseases attacking wheat. In Denmark, only a few specific fungicides are available for controlling PM and the use of resistant cultivars is often recommended. In this study, two Chinese wheat landraces (LR) and two synthetic hexaploid wheat lines were used as donors for creating four nested association mapping populations with a total of 717 individual lines in the hope to identify new powdery mildew resistance genetic variants (Table 1). These lines and the nine parental lines used to create the populations were genotyped using a 20K Illumina SNP chip which resulted in 8902 segregating single nucleotide polymorphisms for assessment of the population structure and whole genome association studies. The largest genetic difference among the lines were between the donors and the elite cultivars, the second largest genetic difference were between the different donors, a difference that was also reflected in differences between

the four nested association mapping populations. The 726 genotypes were phenotyped for powdery mildew resistance in 2017 and 2018. A high PM disease pressure was observed in both seasons, with severities ranging from 0 to >50%. Whole genome association studies for genetic variation in powdery mildew resistance in the individual populations revealed a significant region on chromosome 2B and 2D in each of the four populations. A combined analysis of all the populations confirmed this result and most associated markers explained 42% of the variation in powdery mildew resistance. This study provides both knowledge about the wheat resistance to powdery mildew as well as molecular tools and plant material that can be utilised in marker-assisted selection. Additionally, the four populations produced in this study are highly suitable for association studies of other traits than powdery mildew resistance.

Table 1: Pedigree of the four different NAM populations (Donor lines are indicated in bold)

Population	Pedigree	Number of lines
1	Chinese LR I / Torp // NOS 14095.23 /3/ Capricorn /4/Sheriff	184
2	Synthetic I / Nakskov // Capricorn /3/ Torp /4/ Sheriff	181
3	Synthetic II / 2*Torp // Capricorn /3/ Sheriff	178
4	Chinese LR II / Nakskov // Torp /3/ Capricorn /4/ Sheriff	174
Total		717

Cross the best with the best? On the genomic selection of parents and crosses

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Creating new genetic variation by crossing two or more parents is the initial and often most important step in many plant breeding programs. The choice of parents and predicting the outcome of specific crosses is accordingly of utmost interest in applied plant breeding programs in order to maximize their genetic potential.

Different classical and new concepts for a phenotypic and genomic cross prediction were thus compared among each other in this study by using several simulations as well as an empirical experiment. A strong negative trade-off between short-term and long-term selection gain could be observed in the conducted simulations across several breeding cycles, where methods that achieved the highest short-term selection gain did also lead to a rapid decline in genetic variance, impeding further genetic progress. Notwithstanding, a genomic cross prediction index that aimed to compromise these two factors showed an equal performance as the best established concepts in the short-term, but surpassed previously suggested methods in their long-term selection gain.

The different cross prediction methods were subsequently empirically validated in a field experiment, in which prediction abilities for differentiating crosses were medium to high ($r = 0.35-0.89$) depending on the complexity of the studied traits Fusarium head blight resistance, anther retention, and flowering date. The simulations and empirical study suggested thus that shortening breeding cycles and achieving a higher selection gain in comparison to phenotypic selection is feasible. It was however pivotal to maintain a close genetic distance between training and selection population throughout multiple cycles of selection.

Keywords

Fusarium head blight · genetic diversity · genomic prediction · *Triticum aestivum*

***Triticum militinae* – secondary gene pool - source of new genes suitable for bread wheat gene pool enrichment**

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Triticum militinae belongs to the group of tetraploid Timopheev's wheats (GGAA) that originated from hybridization events of diploid progenitors different to the emmer wheats (BBAA). Crosses between *timopheevii* and emmer wheats result in sterile offsprings in which fertility has to be restored by several backcrosses. Recently, a cross between *T. militinae* and a spring bread wheat cultivar 'Tähti' yielded an introgression line 8.1 with eight introgression segments identified on seven chromosomes. The line 8.1 has altered spike architecture, improved powdery mildew resistance in both seedling and adult stages and altered flowering time. The major powdery mildew resistance locus was high-density mapped and candidate genes were identified. A new allele of the *Vrn1* gene, the *VRN-A1f-like* allele, was characterized as source of the flowering time variability.

Keywords

Flowering · gene introgression · powdery mildew · resistance breeding · vernalization

Acknowledgments

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References

- Abrouk M, Balcárková B, Simková H, Komínková E, Martis MM, *et al.* (2017) The *in silico* identification and characterization of a bread wheat/*Triticum militinae* introgression line. *Plant Biotechnol J* 15: 249-256. DOI: 10.1111/pbi.12610
- Ivaničova Z, Jakobson I, Reis D, Šafář J, Milec Z, *et al.* (2016) Characterization of new allele influencing flowering time in bread wheat introgressed from *Triticum militinae*. *New Biotechnol* 33: 718-727. DOI: 10.1016/j.nbt.2016.01.008
- Jakobson I, Peusha H, Timofejeva L, Järve K (2006) Adult plant and seedling resistance to powdery mildew in a *Triticum aestivum* × *Triticum militinae* hybrid line. *Theor Appl Genet* 112: 760-769. DOI: 10.1007/s00122-005-0181-2
- Jakobson I, Reis D, Tiidema A, Peusha H, Timofejeva L, *et al.* (2012) Fine mapping, phenotypic characterization and validation of non-race-specific resistance to powdery mildew in a wheat-*Triticum militinae* introgression lines. *Theor Appl Genet* 125: 609-623. DOI: 10.1007/s00122-012-1856-0
- Janáková E, Jakobson I, Peusha H, Abrouk M, Škopová M, *et al.* (2018) Divergence between bread wheat and *Triticum militinae* in the powdery mildew resistance *QPm.tut-4A* locus and its implications for cloning of the resistance gene. *Theor Appl Genet*, in press. DOI: 10.1007/s00122-018-3259-3
- Järve K, Jakobson I, Enno T (2002) Tetraploid wheat species *Triticum timopheevii* and *Triticum militinae* in common wheat improvement. *Acta Agron Hung* 50: 463-477. DOI: 10.1556/AAgr.50.2002.4.9

One hundred years of wheat varieties: breeding progress as measured by grain yield, agronomic traits and nitrogen efficiency

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The grain yield obtained from a field is the result of complex interactions between the variety's performance, seed quality, soil quality, previous crop, weather conditions, disease pressure, production system and crop management. Wheat grain yield measured across the whole Austrian production area has not shown an increasing trend since 1992. This is due to a various reasons, including aspects of crop management, unfavorable weather conditions in several consecutive years, the increase of organic wheat acreage and changes in agricultural policy. A lack of breeding progress has also been suggested as factor.

Eleven conventional winter wheat trials were carried out without fungicide use from 2014/15 to 2018/19. The sites are located in the Pannonian northeastern plains and hills (Großnondorf) and the northwestern Waldviertel region (Schönfeld) of Lower Austria, as well as in the Alpine foothills (Bad Wimsbach-Neydharting) of Upper Austria. The sites clearly differ with respect to soil types and climatic conditions. The cultivars were replicated thrice, and the plot size varied from 10 to 11.8 m². The seed density was 300 or 380 germinable grains per m². The trial sites were fertilized with mineral nitrogen (84 to 184 kg ha⁻¹), and growth regulators were occasionally used. In total, the trials were carried out with 20 varieties that have been used in Austrian agriculture since the 1920s.

At Großnondorf, the varieties 'Bernstein' (registered in 2013), 'Brucker Harrachweizen' (1955-1963), 'Capo' (1989), 'Energio' (2009), 'Extrem' (1967-2001), 'Midas' (2008) and 'Perlo' (1978-2001) were compared. The varieties evaluated in Bad Wimsbach-Neydharting and Schönfeld were 'Belmondo' (1997-2016), 'Christian' (1960-1976), 'Diplomat' (1966-1985), 'Frisky' (2014), 'Ikarus' (1983-1998), 'Reichersberger Winterkolbenweizen' (1949-1961), 'Siegfried' (2014) and 'Spontan' (2014). Moreover, some selections from landraces which were cultivated before 1930, *i.e.* 'Otterbacher', 'Sipbachzeller Linie a' and 'Sipbachzeller Linie c' were included. 'Loosdorfer Austro Bankut Grannen' (1948-1968) was included in both trial series.

The extreme differences in plant height, ranging from 91 cm for 'Frisky' to 149 cm for 'Sipbachzeller Linie c', were considered in the randomization of the lattice design as much as possible. The two trial series included 9 and 12 genotypes. The average grain yield in the Pannonian region ranged from 53.3 dt ha⁻¹ for 'Brucker Harrachweizen' to 84.9 dt ha⁻¹ for 'Midas'. In the the western trial series grain yield varied between 51 dt ha⁻¹ for 'Otterbacher' to 93.9 dt ha⁻¹ for 'Siegfried'.

Breeding progress was assessed by regression analysis of the mean variety grain yield on the year of registration. The analysis did not consider ageing effects, which lower the productivity of a variety as its age increases due to a gradual reduction in disease resistance. It can be assumed that this potential loss of disease resistance has already occurred in most of the varieties evaluated.

Breeding progress was low from 1920 until the end of the 1940s. During this period, grain yield increased by less than 0.20 dt ha⁻¹ and year. An increase of 0.46 dt ha⁻¹ per year was measured for the wheat varieties registered between 1948 and 2014 and which were evaluated in the Alpine foothills and in the Waldviertel area. With regards to high breadmaking quality wheat that was grown in the Pannonian region, the observed annual increase in yield was about 0.38 dt ha⁻¹.

This increase in yield was mainly due to an increase in the number of grains per spike or grains harvested per unit area. No clear trend was observed regarding the number of ears per unit area and thousand grain weight.

The results show that the yield stagnation that has been observed in agricultural practice is not due to a lack of genetic improvement. Plant height of the tested varieties decreased by 38 to 68 cm in the western production areas and by 19 to 38 cm in the Pannonian region. This decrease was associated with a significant decrease in lodging. In the trial series carried out in the western areas, serious lodging was occasionally observed in older varieties, which may have affected their grain yield. As a result, the genetic trend is probably somewhat overestimated.

More modern wheat varieties are less susceptible to certain leaf diseases, especially to powdery mildew, leaf rust and yellow rust. The parameter of grain protein yield was used to describe nitrogen use efficiency. Although the protein content of modern varieties is significantly lower, they use the provided nitrogen more efficiently than older varieties.

Keywords

Disease resistance · genetic improvement · nitrogen use efficiency · plant height · protein content · *Triticum aestivum* · winter wheat

References

Ahlemeyer J, Friedt W (2011) Entwicklung der Weizenerträge in Deutschland - Welchen Anteil hat der Zuchtfortschritt? Tagungsband 61. Tagung der Vereinigung der Pflanzzüchter und Saatgutkaufleute Österreichs 2010, pp 19-23. LFZ Raumberg-Gumpenstein, Irdning.

Ahrends HE, Eugster W, Gaiser T, Rueda-Ayala V, Hüging H *et al.* (2018): Genetic yield gains of winter wheat in Germany over more than 100 years (1895-2007) under contrasting fertilizer applications. *Environ Res Lett* 13: 104003. DOI: 10.1088/1748-9326/aade12

Laidig F, Piepho HP, Drobek T, Meyer U (2014) Genetic and non-genetic long-term trends of 12 different crops in German official variety performance trials and on-farm yield trends. *Theor Appl Genet* 127: 2599-2617. DOI 10.1007/s00122-014-2402-z

Piepho HP, Laidig F, Drobek T, Meyer U (2014) Dissecting genetic and non-genetic sources of long-term yield in German official variety trials. *Theor Appl Genet* 127: 1009-1018. DOI 10.1007/s00122-014-2275-1

Unmask the breeding value of genetic resources of barley with a hybrid strategy

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Broadening the genetic base of today's elite breeding programs with untapped favorable alleles for grain yield is crucial to guarantee further breeding success. However, the breeding value of genetic resources is often masked due to the absence of major adaptation genes for important agronomic traits like stem strength. In our study, we performed a proof of concept of a hybrid strategy to obtain unbiased estimates of breeding values for barley genetic resources.

In total, 21 barley genetic resources were selected and by crossing them to elite tester lines, 25 three-way hybrids were produced. These were evaluated together with their parental elite tester lines as well as eight commercial hybrids for important agronomic traits in replicated field trials in Bad Salzflen and Spickendorf (both in Germany) over the years 2017 and 2018. In addition, the genetic resources as well as the elite tester lines were genotyped for 5,562 SNPs.

Two 3-way-hybrids containing genetic resources showed a competitive yield level in comparison with the commercial hybrids. In addition, the phenotypic data analyses revealed that the hybrid strategy facilitated to identify promising barley genetic resources by substantially improving lodging resistance as a central trait for standard yield trials. Combining the results of the molecular and phenotypic data analyses highlighted the potential to boost the diversity of the elite breeding pool by a targeted introgression of genetic resources into the male and female heterotic pools. We propose a large-scale application of the hybrid strategy profiting from the available genomic resources of entire genebank collections and to use the toolbox of genome-wide predictions to support a targeted choice of accessions with high value for barley breeding.

Keywords

Genetic-diversity · grain yield · *Hordeum vulgare* · hybrid breeding

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References

- Longin CFH, Reif JC (2014) Redesigning the exploitation of wheat genetic resources. *Trend Plant Sci* 19:631-636. DOI: 10.1016/j.tplants.2014.06.012
- Sommer L, Spiller M, Stiewe G, Pillen K, Reif JC, Schulthess AW (2020) Proof of concept to unmask the breeding value of genetic resources of barley (*Hordeum vulgare*) with a hybrid strategy. *Plant Breed*, in press. DOI: 10.1111/pbr.12795

Harnessing Brazilian germplasm for the improvement of resistance to Northern corn leaf blight (NCLB) in European maize programs

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Northern corn leaf blight (NCLB), caused by the ascomycete *Setosphaeria turcica*, is one of the most devastating leaf pathogens in maize (*Zea mays* L.). Yield losses can reach up to 63% causing a drastic decrease in farmers' income. Improved host plant resistance is the most sustainable way to defeat this food security threat. Exploiting exotic germplasm is a promising approach to identify novel quantitative trait loci (QTL) for disease resistances in crops, but few studies have been conducted for NCLB resistance in maize. Hence, our study aimed to identify QTL underlying NCLB resistance in two multi-parent QTL mapping populations and to explore the potential use of resistant Brazilian lines as resistance sources for European breeding.

Two resistant Brazilian lines were crossed with five European double haploid (DH) lines to establish five bi-parental families with common parents comprising 484 DH lines. The lines were evaluated in Europe (France, Germany and Italy) and in Brazil for their

testcross performance for NCLB resistance (Figure 1) at six environments (=combination of years and locations) yielding significant genetic variances ($p < 0.01$ to $p < 0.001$) and high entry-mean heritabilities (0.72 to 0.91).

Connected linkage mapping was conducted with the R package mppR. Succinctly, multiple bi-parental populations that were connected through the resistant Brazilian line were analyzed jointly by composite interval mapping. Genomic predictions were conducted by ridge-regression BLUP with the R package rrBLUP. Briefly, two genomic selection analyses were conducted. Firstly, populations connected through the resistant Brazilian line were analyzed jointly in a five-fold cross-validation. Secondly, populations were allocated in different groups according to their genetic relationship. For each group, 60 individuals were included in the training set and the remaining in the validation set. The observed and predicted NCLB values were correlated through the Pearson's correlation

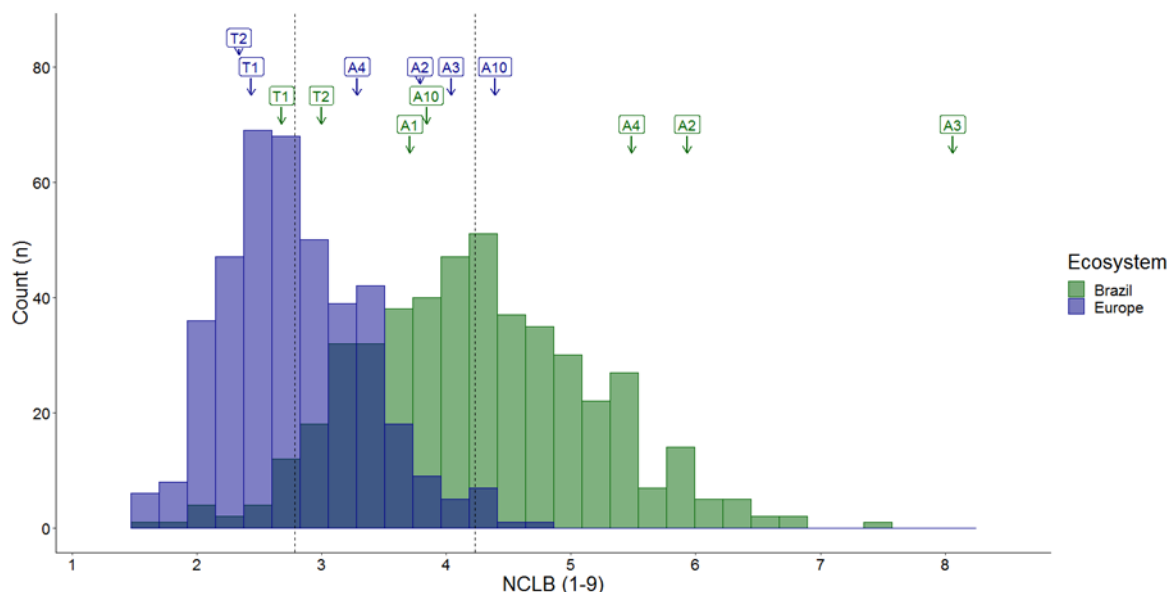


Figure 1: Phenotypic distribution of 484 DH lines tested as testcrosses for NCLB resistance in a total of six environments in Brazil and Europe. The abbreviation "T" stands for tropical Brazilian parents (resistant) and "A" for adapted European parents (susceptible)

coefficient to obtain the prediction abilities. All analyses were conducted in R environment. In addition, we propose a rapid genomic selection scheme for the introgression and integration of major QTL from genetic resources to European breeding programs.

Jointly, we identified nine QTL explaining in the simultaneous fit between 14% and 35% of the total phenotypic variance. Two QTL identified in Europe were also detected in Brazilian environments indicating the stability of these QTL in contrasting ecosystems. Furthermore, most of the QTL identified in the European environment originated from the Brazilian germplasm.

Cross-validated accuracy from genomic prediction yielded about 70%, when including all populations connected by the same resistant Brazilian line. When dividing the connected populations in different genetic relatedness groups we observed that accounting for population relatedness in the training set increases the prediction ability.

Collectively, our study illustrates the potential use of tropical resistance sources to increase NCLB resistance level in European breeding programs as well as the potential use of genomic approaches to assist NCLB resistance breeding programs especially when accounting for population structure.

Keywords

Genomic selection · resistance breeding · *Setosphaeria turcica* · test cross performance · *Zea mays*

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References

- Galiano-Carneiro AL, Miedaner T (2017) Genetics of resistance and pathogenicity in the maize/*Setosphaeria turcica* pathosystem and implications for breeding. *Front Plant Sci* 8: 1490. DOI: 10.3389/fpls.2017.01490
- Endelman JB (2011) Ridge regression and other kernels for genomic selection with R package rrBLUP. *Plant Genome J* 4: 250-255. DOI: 10.3835/plantgenome2011.08.0024
- Endelman JB, Jannink J (2012) Shrinkage estimation of the realized relationship matrix. *G3 (Bethesda)* 2: 1405-1413. DOI: 10.1534/g3.112.004259
- Garin V, Wimmer V, Borchardt D, Malosetti M, van Eeuwijk F (2018) mppR: Multi-parent population QTL analysis. R package version 1.2.0
- Whittaker JC, Thompson R, Denham MC (2000) Marker-assisted selection using ridge regression. *Genet Res* 75: 249-252. DOI: 10.1017/S001667239900462

Basic research in service of applied agriculture and breeding - Applied laboratory of IEB in Czech Republic

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The Applied Laboratory for Agricultural Research is the first workplace of the Czech Academy of Sciences that links scientists with breeders and farmers. It was established under the Food for Future Program of the AV21 Strategy in 2017 and is run by the Centre of Plant Structural and Functional Genomics of the Institute of Experimental Botany AS CR in Olomouc. The main objective of the Applied Laboratory is mediating the latest research results and methods to breeders and agricultural practice in general. One of the main but difficult goals is facilitation of food security in the scope of a growing world population and contributing to food self-sufficiency at national level within the changing climate conditions. We are a world-recognised workplace for sorting of plant chromosomes and their downstream application, including sequencing and data analysis of main crops. We have developed original methods that have helped to break down complex genomes of wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and pea (*Pisum sativum*).

Our team of highly specialized experts offer expertise, professional consultations, workshops, seminars and practical courses for public and private organizations and breeders. We can analyse

samples using various advanced state-of-the-art instruments and methods. The most common are flow cytometry analyses, especially ploidy determinations, cytogenetic analysis, genotyping using different types of DNA markers, NGS sequencing and optical mapping.

This was already applied on different crops, for example: development of genotyping platforms for red clover (*Trifolium pratense*), pea, potatoes (*Solanum tuberosum*), barley, grass and cherry trees to determine the genetic architecture of important agronomic traits in the frame of Biotechnological Centre for Plant Genotyping project. Other examples of fruitful collaboration with organizations involved in the breeding of crops, utility and ornamental plants are presented.

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Breeding for grassland in Austria: a review and outlook

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Forage plant breeding has a long history in Austria. Already 130 years ago, first attempts were made to adapt valuable fodder grasses to the climate, especially with regard to their suitability for seed mixtures in Alpine grassland areas. In 1890, Theodor Ritter von Weinzierl, director of the *Samenkontrollstation der k. k. landwirtschaftlichen Gesellschaft*, the first experimental station was established on the Sandlingalm in 1890. In several publications, Weinzierl summarized his work on the 'climate adaptation' of different origins of forage plant species of the entire Austrian Empire for Alpine grassland production. Soon afterwards, the 'Kraglgut' was established near Bad Mitterndorf, a research station on pasture management and the selection of single plants of various forage grasses.

After Weinzierl's early death in 1917, these experiments were only continued extensively, the two experimental stations were abandoned in the 1930s and the technical agendas were centralised in the *Reichsforschungsanstalt für alpine Landwirtschaft* in Admont. In 1955, the then *Bundesversuchsanstalt für alpine Landwirtschaft* was moved with the entire staff to Gumpenstein.

In 2004, the research and education centres in Gumpenstein and Raumberg, respectively, were merged to the *Höhere Bundeslehr- und Forschungsanstalt für Landwirtschaft (HBLFA) Raumberg-Gumpenstein*.

Breeding activities with forage plants were resumed in the 1960s, with farm varieties of red clover collected primarily by Giselher Schechtner in southern and eastern Styria, from which the first variety of 'Gumpensteiner Rotklee' was bred and finally released in 1974. At the end of the 1980s, another variety, the hybrid ryegrass 'Gumpensteiner' was registered. From 1989 onwards, the breeding activities for a wider range of forage plants were intensified and all agendas were taken over by the author. As a first step, an intensive collection of suitable grassland stocks was built up out to obtain suitable genetic material for further activities. From the beginning it was an important objective to work with regional Austrian genetics as far as possible. Over the next 20 years, intensive work was carried out on a selected range of forage plant species where qualitative and/or quantitative deficiencies were observed during the official Austrian VCU trials.



Figure 1: Theodor Weinzierl and single plants of *Festuca rubra* at Kraglgut in 1909

Table 1: Selected species of permanent grassland and the most important current objectives in forage plant breeding

Species	Permanent meadow	Permanent pasture
Perennial ryegrass	drought stress, winter hardiness (<i>Typhula</i> , <i>Monographella</i>), persistence, rust resistance, Drechslera, Ramularia	winter hardiness (<i>Typhula</i> , <i>Monographella</i>), sward density, persistence, variety trials
Cocksfoot	usage elasticity, drought stress	—
Timothy	Early ripening, multi-cut suitability	—
Kentucky blue grass	rust resistance, establishment, yield, drought stress	establishment, sward density, yield, persistence
Meadow fescue	<i>Drechslera</i> (net blotch disease), multi-cut suitability	persistence
Bird's-foot trefoil	yield, share of condensed tannins	—
Red clover	anthracnose (<i>colletotrichum trifolii</i>), protein content	pasture suitability
White clover	cyanogenic glycosides (hydrocyanic acid content)	—
Festulolium	variety trials	—
Tall fescue	variety trials, digestibility	soft leaves, tillering
Sainfoin	yield, share of condensed tannins	—

During this period a total of 11 varieties of 9 different grasses and legumes were registered. The breeding objectives were defined in very different ways. For example, resilience and high digestibility in cocksfoot (*Dactylis glomerata*), winter hardiness in English ryegrass (*Lolium perenne*), late maturity in meadow foxtail (*Alopecurus pratensis*), low content of calicinogen active substances in golden oat grass (*Trisetum flavescens*) or also resilience and yield in birdsfoot trefoil (*Lotus corniculatus*) or bentgrass (*Agrostis capillaris*). The varieties bred are all propagated in Austria on an area of currently more than 700 ha and exclusively used in quality seed mixtures recommended by the Austrian Association of Grassland and Livestock Farming (<https://gruenland-viehwirtschaft.at>).

Due to rapidly changing climatic conditions and the increasing intensification of grassland production, breeding in Gumpenstein is also facing new challenges. In addition to the important breeding goals of health, resilience and quality yield, improvements such as resistance to new diseases or drought stress are becoming increasingly important. To this end, a close cooperation between Central European breeders is being established, where germplasm and know-how are exchanged. A list of the most important breeding goals is shown in Table 1.

In recent years, in cooperation with the grassland extension services of the Chambers of Agriculture, genetic material has been collected from commercial grassland which, despite multi-cut management, has never been reseeded and should, therefore, still

contain valuable regional genetics. An increasing problem is caused by the genetic drift of regular reseeded commercial grassland species into the original genetics of the still unaltered wild relatives. This problem is becoming increasingly noticeable. Therefore, a project was initiated to secure the original genetics in the in-house grassland gene bank. All these activities are aimed to ensure that future generations will also have access to regional genetic material, whether for breeding activities or for the protection or conservation of the original genetic biodiversity (<https://gzert.at>)

References

- Krautzer B (2015) Pflanzenzüchtung und Saatgutvermehrung. Bericht über die 21. Wintertagung, 29-30. Jänner, Aigen, pp 29-32. HBLFA Raumberg-Gumpenstein, Irdning & Ökosoziales Forum, Wien.
- Krautzer B, Graiss W (2005) Entwicklung und Organisation der Züchtung und Saatgutwirtschaft von Futterpflanzen in Österreich. Bericht über das 11. Alpenländisches Expertenforum, 27.-29. Juni, pp 1-5. HBLFA Raumberg-Gumpenstein, Irdning.
- Krautzer B, Graiss W (2015) Futterpflanzenzüchtung und Generhaltung - was braucht das Grünland? Bericht über das 20. Alpenländisches Expertenforum, 1.-2. Oktober, pp 92-98. HBLFA Raumberg-Gumpenstein, Irdning.

Breeding for drought tolerance in perennial ryegrass

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Abstract

Global climate change is more and more affecting perennial ryegrass (*Lolium perenne* L.) yields by temporal drought periods occurring more frequently. To improve recovery after drought by breeding approaches, segregating biparental populations were phenotyped in a two-location rain-out shelter experiment for two years. Plants were genotyped with a 3.6 k Illumina SNP chip and the single plant metabolome was analysed with a nuclear magnetic resonance (NMR) spectroscopy approach. Genome-wide association mapping identified a number of genomic regions associated with recovery after drought. Genomic prediction abilities for cumulative stress tolerance index reached up to 0.58, whereas prediction based on up to eight metabolites achieved abilities of up to 0.7. Both approaches are promising for enhancing drought tolerance in perennial ryegrass.

Keywords

genetic mapping · genomic prediction · *Lolium perenne* · metabolite analysis · recovery after drought

Introduction

Global climate change will have an enormous impact on plant production in middle Europe with weather extremes occurring more often like increased average temperature but also changes in temporal distribution of precipitation. The years 2018 and 2019 with extensive drought periods gave a first impression of what could be “normal” climate conditions within the next decades. Perennial ryegrass (*Lolium perenne* L.), one of the most important grass species in permanent grassland and forage cropping in Europe, will be particularly affected by temporal drought periods due to limited drought tolerance caused by, e.g., shallow rooting. For example, in the year 2018, yield losses in grassland of up to 40 % were reported for many regions in Germany.

However, genotypic variation for recovery after drought was observed for perennial ryegrass (Bothe *et al.* 2018). To accelerate breeding of new varieties with improved drought tolerance the

presented work aimed to combine different innovative selection methods based on genomics and metabolomics approaches.

Material and Methods

Six perennial ryegrass clones out of the diploid material group with divergent drought stress response (tolerant, medium tolerant, medium susceptible and susceptible) were intercrossed to establish 14 segregating biparental populations comprising 140 individuals each in the combinations shown in Figure 1.

In the years 2017 and 2018 the plant material was phenotyped in a two-location rain out shelter experiment in a randomized block design. Each family was divided in four blocks comprising 35 individuals, complemented by five of ten possible standard clones. Experimental sites were located in Freising/Pulling (Southern Germany) and Malchow/Poel (Northern Germany). Additionally plants were phenotyped under natural precipitation each half of them distributed at the two experimental sites. Watering of the plants in the rainout shelter was withheld in late spring and in midsummer and plants were kept below permanent wilting point for up to four weeks followed by a recovery period of at least six weeks. Recovery of plants after rewatering was visually scored as biomass formation (0 = dead plant, 1 = lowest to 9 = max. biomass) complemented by recording single plant yield.

A membership function value of drought stress tolerance (MFVD) was calculated according to Chen *et al.* (2002) based on a stress tolerance index (STI; Fernandez 1992). For metabolomics analysis leaf samples were taken in 2017 and 2018 before the first drought stress was applied and 10 days after the first cutting date to ensure the sampling of comparable leaf tissue. Samples were put on dry ice immediately and freeze-dried after transport to the lab. Four populations (K07, K10, K12 and K13) were genotyped using the 3.6 k *Lolium* SNP chip (Illumina Infinium assay; Blackmore *et al.* 2015). Genetic mapping of markers was performed using JoinMap 5 software (van Ooijen 2019). Predictive ability of the genomic breeding scores was calculated based on genomic best linear unbiased prediction using the R package ‘synbreed’ (Wimmer *et al.* 2012).

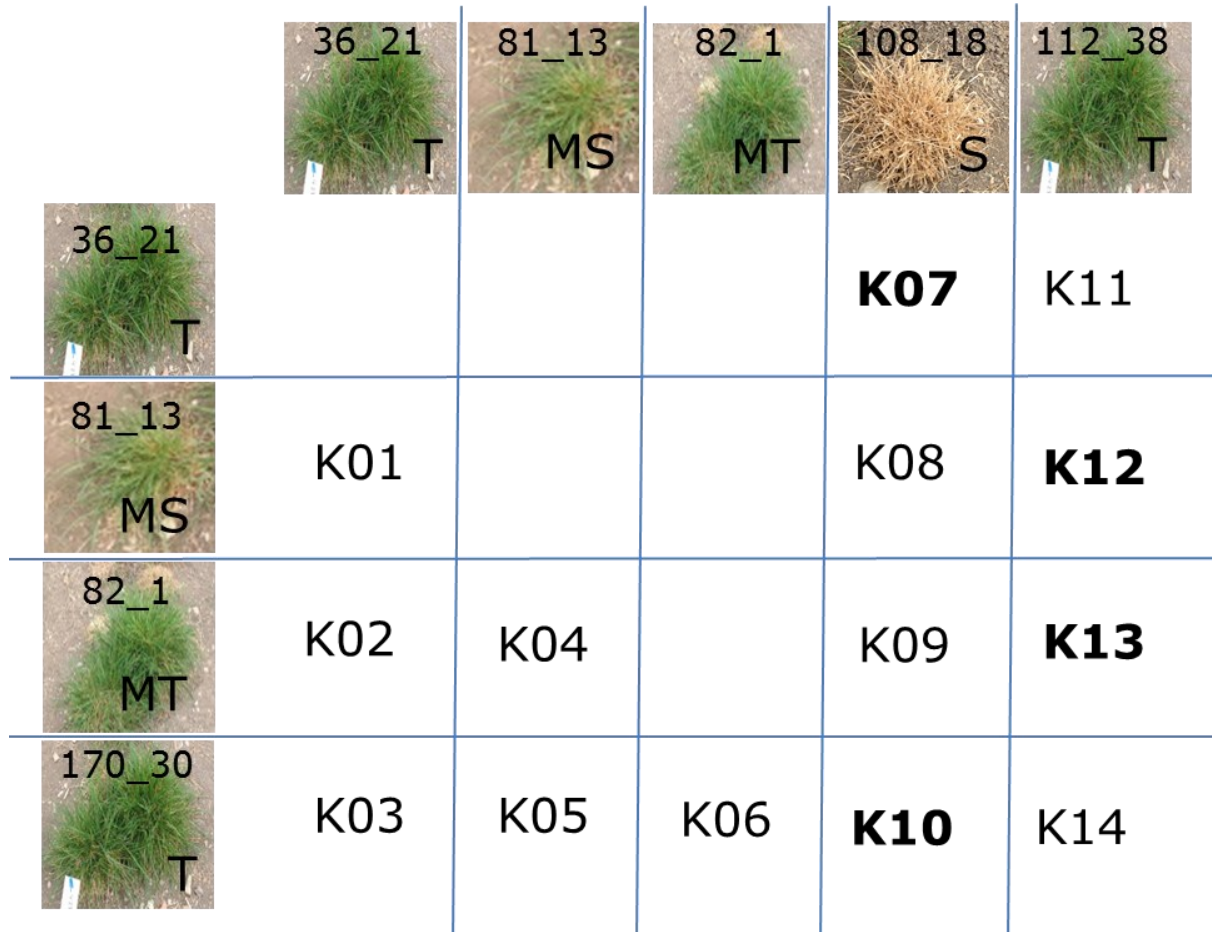


Figure 1: Crossing scheme for biparental perennial ryegrass populations segregating for drought tolerance. Populations in bold were genotyped for genetic analysis. T: tolerant; S: susceptible; MT: moderately tolerant; MS: moderately susceptible.

Results and Discussion

During the two years of experiment an ongoing differentiation of the plant material became visible. Two-location adjusted scorings of biomass formation and single plant yield showed heritability values between 0.27 and 0.72 for the rainout shelter and 0.19 and 0.75 for the well-watered control. Based on this data, MFVD values integrating for the four recovery periods in 2017 and 2018 for each plant were calculated. Figure 2 shows the range of MFVD for each population and the adjusted means of the parental lines.

The data confirmed the wide range of genotypic variation in recovery ability of the plants after drought stress. Whereas susceptible plants showed a severe decline in biomass formation already after the first drought period, from which most of them did not recover properly, tolerant plants showed a good recovery during the rewatering period.

The populations K07, K10, K12 and K13 were genotyped with 3,681 SNP markers assembled on the Illumina iSelect Lolium chip (Blackmore et al. 2015), of which 2,140 markers were informative within this set (560 individuals). Twenty-three genotypes were discarded due to self- or false-pollination. Table 1 shows the prediction abilities for recovery within the four populations.

Genetic maps comprised between 1,261 (K13) and 1,568 (K07) markers spanning 597.3 cM (K13) to 764.4 cM (K10) distributed on

seven linkage groups (LG), except for K13 where no linkage group for LG3 was obtained. Using this information, a first genome-wide association analysis for MFVD was performed. For K07 and K12 marker-trait associations (MTAs) were identified on LG2, whereas the most significant MTAs in K10 and K13 were located on LG1 and LG6, respectively. From this result it is obvious that many different loci (and maybe different underlying physiological mechanisms) are involved in recovery after drought. Further analysis will investigate all 14 populations, connected by common parents, to get a more comprehensive view on candidate regions and their possible combinations within the different crossings. In addition to

Table 1: Genomic prediction abilities for MFVD drought tolerance index in four perennial ryegrass populations. T: tolerant; S: susceptible; MT: moderately tolerant; MS: moderately susceptible

Population	MFVD prediction ability	
	within family	between families
K07 (TxS)	0.364	0.334
K10 (TxS)	0.580	0.546
K12 (TxMS)	0.393	0.447
K13 (TxMT)	0.563	0.559

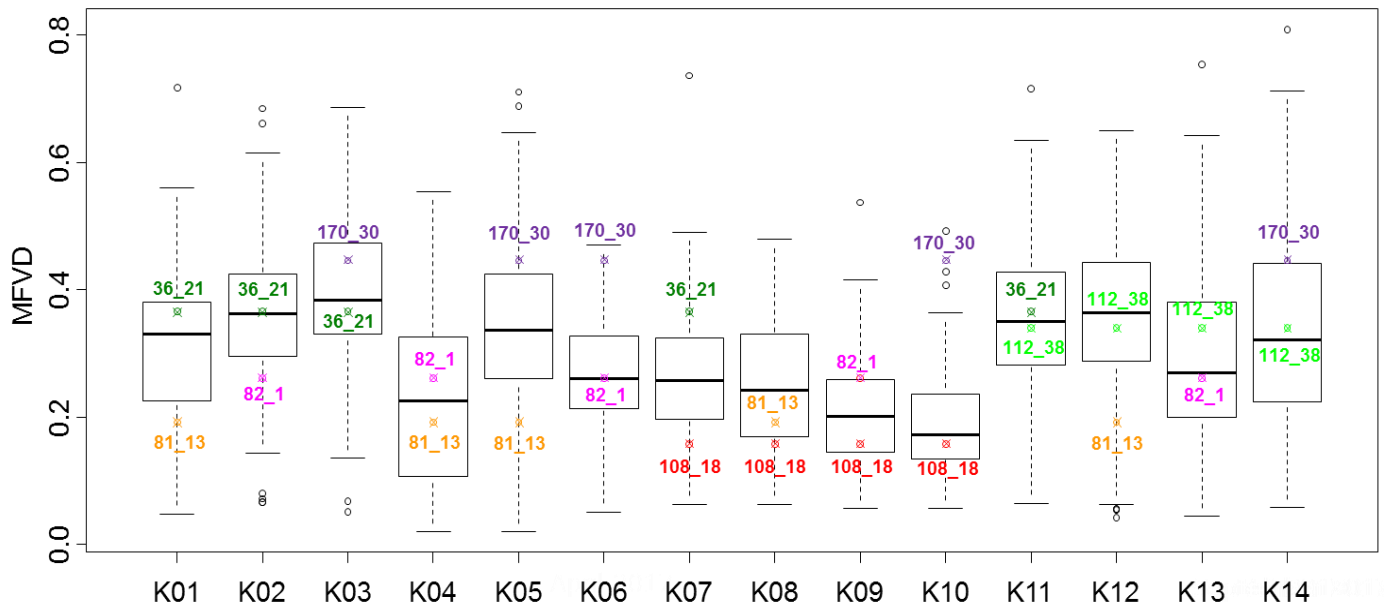


Figure 2: Membership function value of drought tolerance (MFVD) integrating recovery periods over two years. Adjusted means of population parents are given with numbers. T: tolerant; S: susceptible; MT: moderately tolerant; MS: moderately susceptible.

MFVD, intrinsic water use efficiency and leaf elongation rate, both associated with drought tolerance, were investigated for the population K07. The three traits showed overlapping MTAs on LG2, thus suggesting that they are involved in recovery ability.

In a second approach, the metabolome of selected genotypes was analysed using NMR spectroscopy. First results on metabolome-based prediction of drought tolerance are very promising and showed prediction abilities of up to 0.7 taking into account eight of the most relevant metabolites.

After all analyses are completed, a powerful toolbox for selection of drought tolerant perennial ryegrass genotypes will be available. In the next step it is planned to confirm the results in unknown diploid plant material and to extend the methodology to the genetically more complex tetraploid material group.

Acknowledgement

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References

- Blackmore T, Thomas I, McMahon R, Powell W, Hegarty M. (2015) Genetic-geographic correlation revealed across a broad European ecotypic sample of perennial ryegrass (*Lolium perenne*) using array-based SNP genotyping. *Theor Appl Genet* 128:1917-1932. DOI: 10.1007/s00122-015-2556-3
- Bothe A, Westermeier P, Wosnitza A, Willner E, Schum A, Dehmer KJ, Hartmann S (2018) Drought tolerance in perennial ryegrass (*Lolium perenne* L.) as assessed by two contrasting phenotyping systems. *J Agron Crop Sci* 204:375-389. DOI: 10.1111/jac.12269
- Chen X, Min D, Yasir TA, Hu YG (2012) Evaluation of 14 morphological, yield-related and physiological traits as indicators of drought tolerance in Chinese winter bread wheat revealed by analysis of the membership function value of drought tolerance (MFVD). *Field Crops Res* 137:195-201. DOI: 10.1016/j.fcr.2012.09.008
- Fernandez GCJ (1992) Effective selection criteria for assessing plant stress tolerance. *Proceedings of the international symposium on adaptation of vegetables and other food crops in temperature and water stress*. AVRDC, Shanhua, Taiwan, 257–270. DOI: 10.22001/wvc.72511
- Van Ooijen JW (2019) JoinMap® 5.0 software for the calculation of genetic linkage maps in experimental populations of diploid species. Kyazma BV, Wageningen. <http://www.kyazma.nl/>
- Wimmer V, Albrecht T, Auinger HJ, Schön C-C (2012) Synbreed: A framework for the analysis of genomic prediction data using R. *Bioinformatics* 28:2086-2087. DOI: 10.1093/bioinformatics/bts335

Assessment of natural compounds for an eco-sustainable control strategy of Fusarium head blight

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Fusarium head blight (FHB) of wheat is one of the most devastating cereal disease. The causal agents belong to the *Fusarium graminearum* species complex (FGSC), causing head bleaching, necrosis, and shrivelled kernels and mycotoxins accumulation. FHB control strategies are mainly based on synthetic chemicals applications during the flowering stage. Chemicals belonging to Imidazole and Strobilurin are mainly applied to contrast FHB pathogens, but, because their efficiency is highly variable and they are toxic for human, animal and environment, efficient new eco-friendly approaches must be developed.

In this study, we evaluated *in vitro* and *in vivo* anti-fungal activity of different molecules (gallic, ellagic, caffeic acids, chitosan hydrochloride, lignin, oleuropein and esculetin), in single and in combinations, at different concentrations (0.01% - 1%). *In vitro* essays were the following: 96 wells microtiter plate, incorporation, agar diffusion and volatile diffusion assays. Moreover, five different *in vivo* assays were performed, in order to evaluate a putative phytotoxic activity and efficiency against pathogen artificial inoculation. Furthermore, a Real-Time qPCR was performed, in order to understand the role of promising natural compounds on pathogens related genes regulation.

Chitosan hydrochloride 0.5% resulted to be the most versatile and efficient compounds, since it was able to control both spores multiplication and mycelium development, while esculetin and oleuropein resulted to inhibit mycelial growth through volatile diffusion; esculetin showed to reduce roots development. Promising results highlighted that a combination of chitosan hydrochloride and oleuropein is able to contrast *F. graminearum* infection and diffusion as much as chemical controls (tebuconazole and azoxystrobin).

Acknowledgements

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References

- Khan MR, Doohan FM (2009) Comparison of the efficacy of chitosan with that of a fluorescent pseudomonad for the control of Fusarium head blight disease of cereals and associated mycotoxin contamination of grain. *Biol Control* 48: 48-54. DOI: 10.1016/j.biocontrol.2008.08.014
- Kheiri A, Moosawi Jorf SA, Malhipour A, Saremi H, Nikkhah M (2016) Application of chitosan and chitosan nanoparticles for the control of Fusarium head blight of wheat (*Fusarium graminearum*) *in vitro* and greenhouse. *Int J Biol Macromol* 9 Pt A: 1261-1272. DOI: 10.1016/j.ijbiomac.2016.09.072
- Zachetti VGL, Cendoya E, Nichea MJ, Chulze SN, Ramirez ML (2019) Preliminary study on the use of chitosan as an eco-friendly alternative to control Fusarium growth and mycotoxin production on maize and wheat. *Pathogens* 8: 29. DOI: 10.3390/pathogens8010029

Relationship between Fusarium head blight and pigments in wheat grain

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Bread wheat (*Triticum aestivum* L.) is a staple food of 40% of the world's population, hence, the importance in human nutrition and animal feeding is high. Wheat is not just an energy source due to its high starch content, which is easily digestible, but also by its content of phytochemicals with beneficial health effects. These phytochemicals vary among wheat varieties and breeding lines and are accumulated in the aleurone, pericarp or endosperm and may influence the color of the grain (blue, purple and yellow, respectively). Yellow endosperm color is caused by carotenoids, blue and purple grain color by anthocyanins, which are anti-inflammatory, anti-mutagenic, anti-carcinogenic and antibacterial functions. The potential of anthocyanins and carotenoids highly increased due to they may represent alternatives to artificial food colorants. Accumulation of anthocyanin compounds in different plant species is positively related to resistance to pathogens.

The aim of this study was to test the potential of wheat varieties with colored grain against Fusarium head blight (FHB), one of the most devastating fungal diseases of worldwide wheat production. Physiological responses of the flag leaf, which is considered the main photosynthetic organ for grain filling, to FHB during flowering was measured by the rates of transpiration and photosynthesis, respectively. The experiments were performed from 2016 to 2018. Twenty-four spring wheat varieties with colored grain were included in the study, four durum and twenty common wheat varieties. 'Novosibirskaya 67' with white grain color was used as a check. Artificial inoculation of spikes was performed by spraying a spore suspension of *Fusarium culmorum* at Zadoks growth scale 65. The infected spikes were measured for different physiological characteristics (rate of photosynthesis, rate of transpiration). The infection rate was determined by visual symptom scale.

The investigated genotypes showed differences in the rate of transpiration for each year, and only 'Sebesta Blue 2', 'Metzger Blue 2', 'Purple Feed' (*T. aestivum*) and 'ANDW 20A' (*T. durum*) showed significantly different values between stressed plants and the control for all three years. It was observed that 'Purple', 'Konini' and 'Sebesta Blue 2' (*T. aestivum*) showed a lower rate of transpiration in stressed plants for all three years (1.64, 1.76, 1.84 mmol H₂O m⁻² s⁻¹, respectively). 'Purple' showed the lowest mean rate of transpiration for stressed plants (1.64 mmol H₂O m⁻² s⁻¹)

compared to 'Purple Feed' which showed the highest mean rate of transpiration in stressed plants (2.48 mmol H₂O m⁻² s⁻¹). All durum varieties and the control 'Novosibirskaya 67' were in the medium of the range of results.

Mean values of photosynthesis rate indicated significantly different values between control and inoculated plants for all three years and almost all genotypes except 'Sebesta Blue 2', 'Metzger Blue 2' and 'ANK-28A'. Generally, varieties with purple pericarp had in average a higher photosynthesis rate than the red-grained varieties. The wheat genotypes 'Metzger Blue 2', 'Aoi Yu ANK-1B' and the durum genotypes 'ANDW 9A', 'ANDW 9B', 'ANDW 20A/4A' and 'ANDW 20A' showed the lowest mean photosynthesis rate.

In conclusion, durum varieties with colored grain were more susceptible to *Fusarium* infection compared to colored common wheat varieties. This was also confirmed by the lowest mean photosynthesis rate in genotypes of *T. durum*. Contrary, the results of the transpiration rate did not confirm these conclusions. Several genotypes were detected showing good resistance to FHB among the studied colored wheat collection compared to common colored wheat. The results of some genotypes were interesting in our study and the materials appear to be promising.

Keywords

Anthocyanins · grain colour · mycotoxins · wheat

Acknowledgments

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References

Bogdanova ED, Sarbaev AT, Makhmudova KK (2002) Resistance of common wheat to bunt. In: Proceedings of the Research Conference on Genetics. Moscow, Russia, pp 43-44.

Bowen-Forbes CS, Zhang YJ, Nair MG (2010) Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits. *J Food Compos Anal* 23: 554-560. DOI: 10.1016/j.jfca.2009.08.012

Izdebski R (1992) Utilization of rye genetic resources - initial material section. *Hereditas* 116: 179-185. DOI: 10.1111/j.1601-5223.1992.tb00225.x

Kähkönen MP, Heinonen M (2003) Antioxidant activity of anthocyanins and their aglycons. *J Agric Food Chem* 51: 628-633. DOI: 10.1021/jf025551i

Li D, Wang P, Luo Y, Zhao M, Chen F (2017) Health benefits of anthocyanins and molecular mechanisms: update from recent decade. *Crit Rev Food Sci Nutr* 57: 1729-1741. DOI: 10.1080/10408398.2015.1030064

Smeriglio A, Barreca D, Bellocco E, Trombetta D (2016) Chemistry, pharmacology and health benefits of anthocyanins. *Phytother Res* 30: 1265-1286. DOI: 10.1002/ptr.5642

Wang LS, Stoner GD (2008) Anthocyanins and their role in cancer prevention. *Cancer Lett* 269: 281-290. DOI: 10.1016/j.canlet.2008.05.020

Phenomics analysis and risk prediction of Fusarium head blight

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Fusarium Head Blight (FHB) is a disease of small grain cereals reducing grain yield and quality. FHB incidence and severity vary with cereal growing areas, weather conditions and host plant resistance. *Triticum durum* results to be much more susceptible than *T. aestivum* and FHB control strategies are focused on using Imidazole and Strobilurin during the flowering stage. Chemicals efficiency is highly linked to treatment timing, mainly because infection establishment is strictly dependent on plant phenological stage (pre-anthesis) and because of the quick mycelium development through the entire spike. Regarding that, estimation of FHB risk and early detection in field are important goals, since they can accurately help to manage control strategies, in order to reduce and optimize fungicide treatments.

Studying the pathogen epidemiological stages, we individuated its optimal growth environmental conditions, in order to adapt an existing decision support system model to monitor the pathogen in Umbria Region. The epidemiological informations have been organized in a web-based system, called SMARTAGRI, able to generate five different FHB risk levels, on the basis of climate and agronomic informations of four partner farms located in the Umbria Region. In order to establish an innovative FHB early detection method, an experimental plot of *T. durum* cv. Marco Aurelio located in Amelia was subjected to thermal and RGB imaging in association with an Unmanned Aerial Vehicle (UAV), plant samp-

ling to confirm pathogen presence, in-field spike temperature measurements and photosynthesis efficiency evaluations.

After mosaicking, orthorectifying and georeferencing, the first in-field trial revealed the “leopard spot” FHB distribution and an increasing in temperature value in infected spikes, probably due to differential stomatal conductance regulation. A Real-Time qPCR will be performed on a series of 20 key genes, in order to assess the role of stomatal conductance in FHB resistance and a putative differential regulation between infection status and drought stress status.

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RYE-SUS: a transnational approach to support sustainable grain production in changing environments

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Abstract

Architectural traits such as plant height are important targets to improve the yield potential of crops. The transnational project RYE-SUS makes use of hybrid and genome-based precision breeding to increase target-specific selection efficiency and accelerate breeding processes in rye. Within RYE-SUS, semi-dwarf rye will be developed for the first time ever using the male sterility inducing Pampa cytoplasm and the dwarfing gene *Ddw1*. The new genotypes with an optimized harvest index are expected to increase the yield potential mainly by exploiting heterosis as well as by mobilization of a larger part of the total plant assimilates to the

grain, which improves the resource-use efficiency of rye. The genetically reduced gibberellin content of semi-dwarf rye results in new phenotypes to confer lodging tolerance and to strengthen the adaptation of rye to a changing climate. Lodging-resistant genotypes will considerably improve rye production by securing yield quantity and quality, saving cost and energy for grain drying, and reducing environmental impact by forgoing chemical growth regulators. As a further novelty to improve rye health and protection, every single semi-dwarf rye plant will produce pollen in amounts sufficient to minimise the risk of extremely toxic ergot alkaloids in the harvest. A highly diagnostic field phenotyping platform representing target environments for rye production in Europe and

Canada enables to prove the practical potential and to develop and utilize a simulation model of the growth and development of semi-dwarf as well as tall rye under potentially growth-limiting factors such as drought, frost, or nutrient deficiencies.

Keywords

Biodiversity · climate change · dwarfing gene · food security · harvest index · plant height · *Secale cereale* · sustainability

Introduction

The systematic exploitation of heterosis by hybrid breeding is one of the most sustainable innovations in plant breeding. Heterosis was first described in detail by the American botanist and plant geneticist George Harrison Shull (1908). In rye (*Secale cereale* L.), the systematic exploitation of heterosis by hybrid breeding started around 1970 and the first hybrid rye cultivars were released in 1984 (Geiger & Miedaner 2009). The mating of genetically diverse, pure-bred lines that carry certain desirable traits result in uniform offspring that outperform open-pollinating rye cultivars in grain yield and other agronomic traits (Laidig *et al.* 2017).

In the cross-pollinating rye, hybrid breeding is more flexible than population breeding in creating cultivars with specific traits. In 1983, five population cultivars covered 99.5% of the total rye seed multiplication area in Germany with the most popular variety having a share of 63.5% in total area (Barth *et al.* 2004). In 2018, the German Federal Plant Variety Office describes 32 registered winter rye cultivars for grain use in the descriptive variety list (Bundessortenamt 2018). Hybrids represent 72% of these cultivars. The five largest cultivars include two populations and three hybrids which altogether cover 63% of the seed multiplication area (Bundessortenamt 2018). Hybrid breeding, thus, supplements the opportunities to genetically improve this small grain cereal and triggers the increase of intraspecific biodiversity, *i.e.* the genetic component of biodiversity in rye.

The natural genetic diversity in rye was the fundamental basis to achieve a series of technological advances, which facilitated to

establish hybrid breeding. These include self-fertility mutations, which enable the development of inbred lines to capture valuable genetic diversity, nuclear-cytoplasmic gynodioecy to establish a natural, reliable, environmentally friendly and cost-effective production of hybrid seed, effective restorer-of-fertility (*Rf*) genes to enable grain production in CMS-based hybrids, as well as two genetically divergent gene pools, Petkus and Carsten (Hepting 1978), to exploit heterosis. Hybrid breeding has resulted in a substantial breeding progress for yield and quality traits in rye during the last 26 years (Laidig *et al.* 2017). Recently, genome-based prediction of breeding values has been implemented in a hybrid rye breeding program, which is expected to further increase selection gain (Auinger *et al.* 2017).

Despite of these major achievements, major challenges in rye cultivation remain, including, lodging and a much lower harvest index (HI) as compared to wheat. Plant height is a major factor influencing lodging tolerance and counts among the major target traits in rye breeding. Plant height is a quantitative inherited trait with complex genetic architecture in rye (Auinger *et al.* 2017; Miedaner *et al.* 2011, 2012, 2018), rendering the genetic improvement of plant height a challenging task. The dominant dwarfing gene *Ddw1*, that was initially designated *Humilus* (*HI*) (Kobyljanski 1972) and later renamed by Melz (1989), offers a breeder's option to improve lodging resistance and increase the HI of rye. *Ddw1* has been successfully used in breeding of open pollinating cultivars, which may be characterised by increasing frequencies of tall plants, as genotypes carrying the recessive *ddw1* allele are difficult to detected within the seed multiplication process of a semi-dwarf open-pollinating population (Kobyljanski 1988, McLeod *et al.* 2000, Torop *et al.* 2003, Tenhola-Roininen & Tanhuanpää 2010). However, this mutant has not yet been utilized in hybrid breeding programs (Geiger & Miedaner 2009; Miedaner *et al.* 2011, 2012, 2018). We have applied *de novo* transcriptome sequencing and comparative mapping (Braun *et al.* 2019) to establish and use novel markers for the introgression of *Ddw1* into the Petkus pool. Homozygous semi-dwarf as well as near isogenic tall BC₃S₂ lines in the male sterility inducing Pampa (P) cytoplasm are now available to develop semi-dwarf P-type hybrids for the first time ever in rye improvement programs (Figure 1).

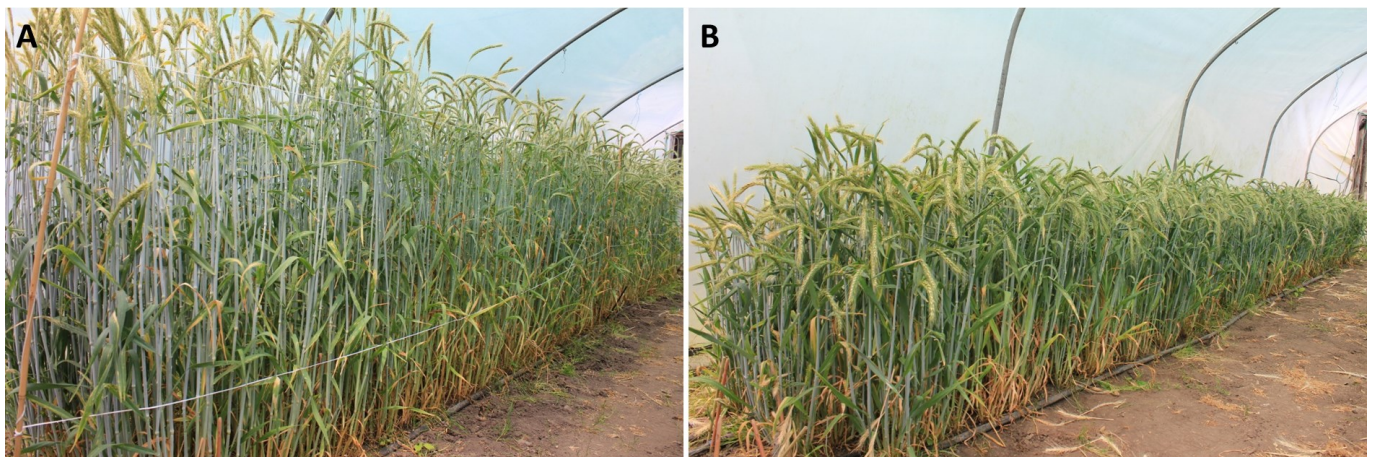


Figure 1: Marker-assisted introgression of *Ddw1* in elite seed parent lines enabled to establish semi-dwarf and near isogenic, tall single cross seed parent lines in the P-cytoplasm.

Enhancing photosynthesis efficiency in rye

Photosynthesis is the primary determinant of crop yield. Enhancing photosynthesis, electron transport and photorespiration has, thus, been accepted as a key target for increasing crop productivity (Simkin *et al.* 2019). Hybrids are characterized by a particularly effective light interception machinery beginning with seedling vigour and including homeostasis of photosynthesis over a broad range of environmental conditions as the basis for a strong source (Blum 2013). Transcriptional profiling in rice revealed, that hybrid breeding enables to increase activity of enzymes involved in carbon fixation pathways and net photosynthetic rate (Song *et al.* 2010). The well established heterotic pattern of rye, therefore, offers a proven option to systematically increase and secure basic grain productivity in terms of converting solar irradiation into biomass on finite arable land without increasing water and fertilizer use.

Likewise and as opposed to wheat, the route for improving grain yield by increasing the HI of rye is not approaching its end. The main sites of photosynthesis are leaves, and plants with leaves of optimal shape and size harvest light more efficient, leading to a rapid growth rate and increased yield. Morphological characters of the plant like the size and shape of leaves as well as their spatial arrangement determine a plants architecture. The importance of the plant architecture on crop yield potential became evident during the Green Revolution, when the genetic improvement of plant height in wheat and rice resulted in an unprecedented increase in agricultural production (Hedden 2003). Similar to wheat, the spike has proven to be supplied with assimilates essentially via the flag leaf (F) and F-1 in semi-dwarf *Ddw1* genotypes, while in their tall equivalents the stem is the main photosynthesis organ (Kobyljanski & Babuzhina 2007). A genetically optimized allocation of dry matter to the grain by means of *Ddw1*, together with the systematic utilization of heterosis, offers a strategy to increase rye productivity that is substantially different from current methods in the genetic improvement of rye and wheat.

Translating fundamental research into climate resilient crops

Ddw1 belongs to the group of gibberellin (GA) sensitive dwarfing genes (Börner & Melz 1988). Noteworthy in this context, it is becoming increasingly evident that the GA class of plant hormones is of pivotal relevance in the response of plants to abiotic stress (Colebrook *et al.* 2014). Positive effects on grain yield, lodging tolerance as well as drought tolerance in tef (*Eragrostis tef* (Zucc.) Trotter) and finger millet (*Eleusine coracana* Gaertn) have been reported as a result of chemically induced GA deficiency (Plaza-Wüthrich *et al.* 2016). In rice, induced mutants of the GA deactivation gene GA2-oxidase 6 (*GA2ox6*) moderately reduced GA concentration and reprogrammed transcriptional networks, leading to reduced plant height, more productive tillers, an expanded root system, higher water use efficiency and photosynthesis rate, and elevated abiotic and biotic stress tolerance (Lo *et al.* 2017). In accordance with these observations the analysis of the Arabidopsis NAC-like GIBBERELLIN SUPPRESSING FACTOR (GSF) showed a novel function in the regulation of gibberellin biosynthesis (Chen *et al.* 2019). The ectopic expression of GSF lacking a transmembrane domain (GSF-TM) caused a dwarf phenotype, which was correlated with the upregulation of *GA2ox2/6* and an increased drought tolerance compared to the wild-type plants.

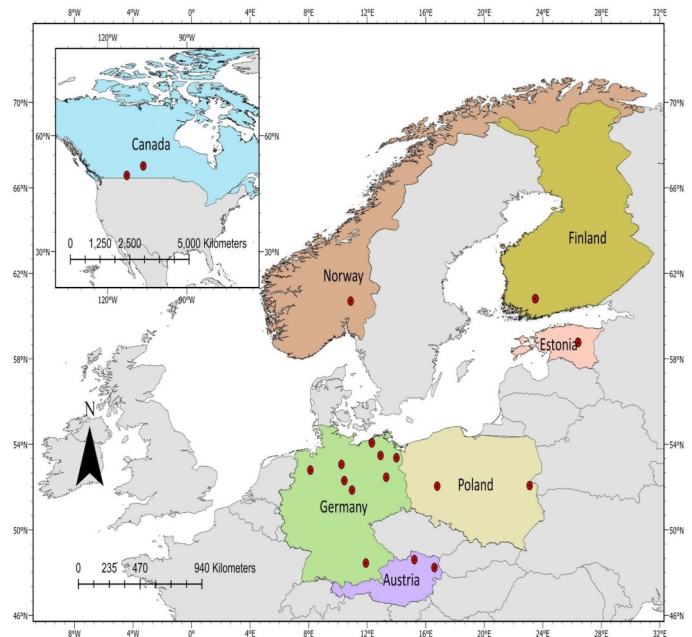


Figure 2: The RYE-SUS phenotyping network.

We have identified *ScGA2ox12* that is up-regulated in semi-dwarf rye and co-segregating with the GA-sensitive dwarfing gene *Ddw1* (Braun *et al.* 2019). The described progress in our understanding of the importance of GA-homeostasis for drought stress tolerance, thus, implicates that the established semi-dwarf seed parent lines most likely confer both, lodging and drought tolerance in rye, as the *Ddw1* mutant alters the GA content in a favourable manner. Interestingly, a root-derived precursor of bioactive gibberellins has recently been described to mediate thermo-responsive shoot growth in Arabidopsis suggesting that root-to-shoot translocation of GA12 enables a flexible growth response to ambient temperature changes (Camut *et al.* 2019). According to this research it can be assumed, that the root signal cannot trigger stem growth in GA-sensitive semi-dwarf rye, at least to the same extend, as in wild-type plants. As a consequence, dry matter in semi-dwarf rye will be allocated to the grain rather than to the stem, even under elevated temperature. The genetic adaptation of rye to a changing climate by means of GA-sensitive semi-dwarf rye is of particular importance for grain production, as rye is mainly cultivated on light soils with low fertility and water holding capacity. Previous research emphasised the improvement of drought stress tolerance in rye an indispensable task. An average drought induced grain yield reduction of 23.8% has been reported for hybrid rye in non-irrigated compared to irrigated regime under natural drought stress conditions (Hübner *et al.* 2013), while up to 57% grain yield reduction was observed in controlled environments under different drought regimes (Kottmann *et al.* 2016). By conducting transnational field trials with semi-dwarf rye in target environments of rye cultivation (Figure 2), RYE-SUS represents an unique opportunity for the proof of concept, that mutants in the GA biosynthesis pathway provide valuable genetic variants to create lodging tolerant and climate-smart crops. In this context, canopy temperature as an indicator of crop water status (Kottmann *et al.* 2013) might be a useful trait to assess and understand the drought-stress response of semi-dwarf rye.

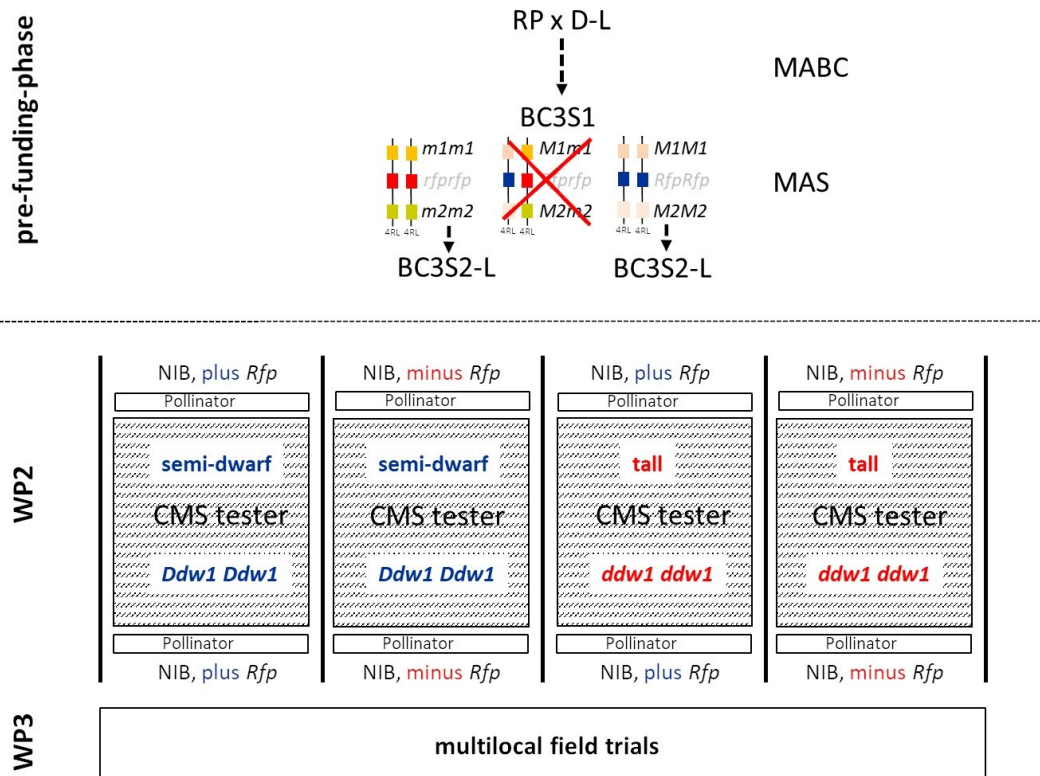


Figure 3: Flow diagram illustrating seed production of semi-dwarf and near-isogenic tall experimental rye hybrids (RP: recurrent parent; D-L: donor line; MABC: marker assisted backcrossing; MAS marker assisted selection; NIB: near isogenic bulk)

A new era in cereal breeding

Ergot is a disease of cereals and grasses caused by fungi in the genus *Claviceps* (Miedaner & Geiger 2015). At anthesis the open, non-fertilized florets of rye enable ergot spores to access the stigma and mimic pollination. CMS-based hybrids with an unsatisfactory restoration level and reduced pollen shedding are notably susceptible to ergot as the fungal spores have no competitors during the infection of the stigmatic tissue (Hackauf *et al.* 2017). The risk of ergot contamination in rye can be particularly high in years with cool and rainy weather during rye flowering which in turn results in high costs for cleaning the harvested seed. *Claviceps purpurea* produces all three major groups of ergot alkaloids: clavine alkaloids, D-lysergic acid and its derivatives, and ergopeptines (Miedaner & Geiger 2015). Because of the toxicity of ergot sclerotia for human and animals, the European Community regulation No. 687/2008 restricts the concentration of ergot bodies to 0.05% in wheat for human consumption. This threshold is commercially used in rye as well. The European directive 2002/32/EC on undesirable substances in animal feed fixes a limit of 0.1% for ergot in feedstuff containing unground cereals. The compliance of thresholds for ergot contamination in the harvest is, thus, critical for a reliable marketing of rye products. As a consequence, ergot infection counts among the economically most important diseases in rye and should be minimized in commercial production. *Rf* genes are of central importance for cereal hybrid breeding, both for achieving maximum seed setting as well as for minimizing ergot infestation. However, hybrids carrying an effective *Rf* gene for the P cytoplasm (*Rfp*) suffer from a significant reduction in grain yield (Miedaner *et al.* 2017). As a consequence of the high yield penalty, a restricted integration of *Rfp* genes from weedy rye in the pollinator gene pool gaining a restorer index of $\approx 50\%$ is considered as

a feasible practice (Miedaner *et al.* 2017). However, rainy weather at flowering time reduces pollen shedding and pollen movement. As wet pollen agglutinates and distributes over short distances only, a restorer index of $\approx 50\%$ may result in insufficient quantities of pollen to combat the fungus adequately. In order to comprehensively reduce the risk of ergot infection in hybrid rye, varieties should be developed with a restorer index of 100%, *i.e.* male fertility restoration is realized for every single plant.

We have recently shown, that the yield penalty associated with a defined donor chromosome segment (DCS) on chromosome 4RL, carrying *Rfp2* or *Rfp4* from forage rye, is less pronounced than a DCS carrying the most prominent *Rfp1* gene from a weedy rye (Hackauf *et al.* 2018). Therefore, natural biodiversity of effective *Rf* genes offer valuable means to minimise the costs of restoration in terms of grain yield. BC₃S₁ single plants have been selected carrying either the elite (E) non-restorer allele or the donor (D) restorer allele from weedy (*Rfp1*) and forage rye (*Rfp2* and *Rfp4*) introgressed in two elite inbred lines using previously developed markers (Hackauf *et al.* 2012) to establish near isogenic homozygous BC₃S₂ lines (NIL), that will be used in RYE-SUS as pollinators for crossing with three male sterile semi-dwarf as well as their near isogenic tall single-cross non-restorer genotypes between isolation walls (Figure 3). The genetic makeup of these hybrids enables to calculate linkage drag effects as the difference ($\Delta E-D$) between the means of individual NIL partners, which either carry the E or the D at the 4RL DCS. Similarly, the effect of *Ddw1* will be determined by pairwise comparisons between semi-dwarf and near isogenic tall genotypes. As a consequence, our research conducted in RYE-SUS will clarify, if the fitness costs of *Rfp* genes can be counterbalanced by using beneficial effects of *Ddw1* on grain yield. This proof of concept will open a new era in breeding

hybrid cultivars with high yield potential and minimized risk of ergot infestation. While the technology of hybrid breeding enables to rapidly develop and precisely evaluate prototypes of new rye genotypes, the knowledge gained will be applied within RYE-SUS for the genetic improvement of open-pollinated cultivars with simple and less expensive breeding procedures and short development cycles as well.

Uncover the hidden half of rye

The main driver enabling rye cultivation on light soils with low fertility and low water capacity is its highly developed root system, which facilitates a very efficient uptake of water and nutrients (Starzycki 1976). The power of rye roots becomes apparent by different observations. The entire root system of a single rye plant consisted of 13,815,672 branches, with a total length of 622 km, a surface area of 401 m², and a total root hair length of 11,000 km (Dittmer 1937, Ryser 2006). Winter rye showed the lowest yield reduction compared to winter wheat, winter barley, and winter triticale, when rainfall was fully excluded by means of rain-out shelters from tillering until harvest (Schittenhelm *et al.* 2014). Rye reduced nitrate leaching at the field scale by up to 93%, followed by barley (87%) and wheat (57%), as compared to the estimated seasonal average of 43 kg ha⁻¹ over the winter fallow period without cover crop (Yeo *et al.* 2014). The increased nitrogen use efficiency (NUE) of rye is mainly attributed to its higher specific root length (Paponov *et al.* 1999). The high NUE of winter rye also enables grain production with the lowest carbon footprint as compared to wheat, triticale, barley and oats (Wojcik-Gront & Bloch-Michalik 2016). Thus, the cultivation of rye contributes to reduce nitrogen losses and greenhouse gas emissions. Both are serious challenges for (i) a sustainable agriculture that should ensure food security for a growing human population as well as (ii) the need to manage the world's continuously increasing demand for energy and fresh water in view of climate change (Sawamoto *et al.* 2005; Philippot & Hallin 2011).

However, there is currently no information available about possible changes in the rooting pattern of hybrid rye cultivars as a result of breeding activities during the last decades. In wheat, the introduction of GA insensitive dwarfing genes caused a reduction of root mass and root length, even though crop productivity was significantly increased (Waines & Ehdai 2007, Subira *et al.* 2016, Voss-Fels *et al.* 2017). It is currently under discussion whether the reduction in plant height does also reduce the water uptake capacity of cereal crops. Zhang *et al.* (2009) suggested that recent semi-dwarf wheat cultivars are even more efficient with regard to water use, because the decrease in total root length was mainly at the expense of root length in the top soil, and led to a higher proportion of roots in deeper soil layers. On the other hand, Waines & Ehdai (2007) assume that the root system sizes of modern, GA insensitive cultivars are sufficient under optimal growth conditions, but insufficient when growth conditions are unfavourable. Root phenotyping, thus, provides an important information for a comprehensive evaluation of GA sensitive, semi-dwarf rye. Root phenotyping can be conducted in controlled environments like tubes and rhizotrons. However, results from controlled environments cannot be easily transferred to field conditions, especially for deep roots. Although direct root sampling in the field is time consuming, it enables an unbiased detection of genetic effects on depth and density of roots (Clarke *et al.* 2017). To uncover the

hidden half of rye, root phenotyping within RYE-SUS will be conducted directly in the field within the first three weeks after anthesis to determine total root length as well as root length density, and root dry weight of semi-dwarf as compared to near-isogenic tall rye up to a soil depth of 150 cm.

New perspectives for rye cultivation in northern latitudes

Winter hardiness gains increasing importance for establishing highly productive rye cultivars in marginal high latitude production regions of Europe and Canada, where continental climates and severe winters require high levels of frost tolerance. Recently, new insights on winter-hardiness in rye has been reported (Bahrani *et al.* 2019). The winter survival rate of both cultivars 'Halo' and 'Kustro' was classified with the highest winter survival score (very high), while the cultivars 'Petkuser Kurzstroh' and 'Petkuser' both belong to the opposite class with very low winter survival rate. Likewise, winter survival of the cultivars 'Carokurz' and 'Carstens' was classified as moderate and low, respectively. These results demonstrate, that desired and indispensable alleles for winter hardiness are available in adapted material of the Petkus pool and may exist at low frequency in the Carsten pool as well. The broad genetic diversity available in both heterotic gene pools may, therefore be useful to identify valuable alleles and mechanisms of winterhardiness in rye.

Tagging of specific genes or defined genomic regions with gene-specific markers enables an efficient introgression of favorable alleles from donor populations in elite material by efficient marker-assisted introgression programs. Frost tolerance (FT) contributes to winter hardiness and is a complex biological process involving at least two main pathways and many additional processes encompassing a large number of genes (Babben *et al.* 2018). In barley and wheat, two major FT loci, frost resistance 1 (*Fr1*) and frost resistance 2 (*Fr2*), were identified on the long arm of the homeologous group 5 chromosomes (Vagujfalvi *et al.* 2003, Francia *et al.* 2004). The *Fr2* locus on chromosome 5R in rye has been reported to improve the recovery of plants after winter (Li *et al.* 2011, Erath *et al.* 2017). Interestingly, none of the analyzed genes residing at the *Fr2* locus affected the survival after winter. This result indicates, that factors beyond the *Fr2* locus might control the outstanding winter hardiness of rye. However, it needs to be considered that phenotypic data on winter hardiness may be influenced by environmental factors that mask genetic differences in FT (Erath *et al.* 2017, Babben *et al.* 2018). Notably, the timing of floral transition is one of the determining factors for FT tolerance in winter wheat, where the timing of the transition from a vegetative to a generative growth stage has been found to be associated with for how long the plants are able to maintain a high level of FT (Fowler & Limin 2004). Genetic diversity in temperature sensing and/or signaling results in differences of leaf initiation and maturation and/or vernalization rates in wheat (Båga *et al.* 2009). Thus, the final leaf number is an additional, yet not studied parameter in rye, that can contribute to unravel the frost tolerance network. Genes increasing the final leaf number may hence be of importance for the development of elite rye germplasm with improved winter hardiness.

The recently published rye reference genome sequence (Rabanus-Wallace *et al.* 2019) facilitates many downstream applications including efficient development of gene specific primers. An in-

depth characterization of candidate genes beyond the CBF regulatory hub (Thomashow 2010) residing at the *Fr2* locus offers a novel opportunity to get insights into the genetic architecture of winter hardiness in rye. Genes involved in the anthocyanin biosynthesis pathway count among our targets, as for the most cold-hardy an overall higher abundance and diversity of anthocyanins has been noted as compared to the less hardy genotypes (Bahrani *et al.* 2019). It needs to be considered that the regulation of anthocyanin biosynthesis is less well-described in rye than in *Arabidopsis* (Shi & Xie 2014), maize (Petroni *et al.* 2014), or rice (Zheng *et al.* 2019). However, genetic diversity in the anthocyanin pigmentation of rye coleoptiles served to identify the six recessive genes *vi1*–*vi6* (Voytkov *et al.* 2015). A novel 20k custom wheat/rye/triticale SNP array, that contains 5k SNPs selected from the rye 600k SNP array (Bauer *et al.* 2017), and bulked segregant analysis (Michelmore *et al.* 1991) enabled us to map these genes on chromosomes 2R, 3R, 4R and 7R (Figure 4). A phenotyping strategy based on a $F_{2:3}$ design and testcross performance in multi-environmental field trials (Hackauf *et al.* 2017) will be used to unravel the relevance of these genes to control winter hardiness in rye.

The way forward: predicting the future of rye

Process-based crop models are essential tools in current agronomic research. They enable simulations of crop growth, development and yield under diverse soil, climate and management conditions (Jones *et al.* 2003). Crop models consider the soil-plant-atmosphere-system and the involved carbon-, water- and nitrogen fluxes. They make it possible to complement and upscale empirical research, and may hence contribute to complement the necessity of expensive field trials. In addition, they enable testing and evaluation of scenarios for future altered climatic, technological or cropping systems related conditions (Munz *et al.* 2014). Crop models may be used to generate missing data in large-scale agronomic and experimental data sets, such as crop phenological information (Dreccer *et al.* 2018) or potential yield levels (van Rees *et al.* 2014). In recent years the application of crop models for climate change impact assessment and development of climate change adaptation strategies (Qin *et al.* 2018) has gained increasing importance. In this context, crop models may also contribute to the development of crop ideotypes for current and future production conditions (Gérardeaux *et al.* 2018), ideotypes that feature necessary phenotypic and genotypic traits in order to optimize yield under given pedo-climatic conditions. Another useful application of crop models is to test and evaluate the introduction of a new crop or crop cultivar to a specific environment in virtual experiments (George & Kaffka 2017). Up to now mainly phenotypic data have been used for model parameterization (Gouache *et al.* 2017). Those data may, as planned in RYE-SUS, be complemented by genetic information on QTL governing growth and development, which could improve the prediction of a genotype's performance in different environments even further (Hwang *et al.* 2017).

A specific challenge regarding models for winter cereals is the simulation of winter survival and hence the remaining field plant cover in spring. The FROSTOL model (Bergjord *et al.* 2008) was developed to simulate course of frost tolerance from sowing on towards spring. The implementation of a spring field plant coverage factor resulted in improved predictions of wheat growth and yield (Bergjord Olsen *et al.* 2018). By quantifying relationships

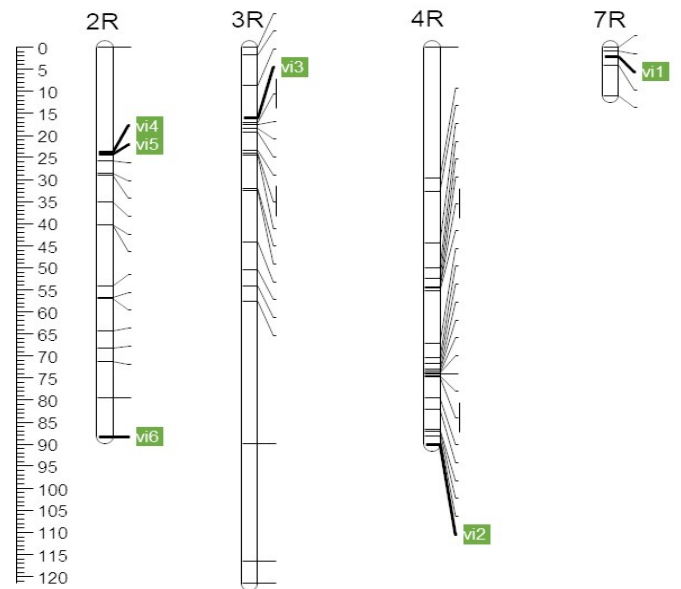


Figure 4: Chromosomal localization of genes controlling anthocyanin pigmentation of rye coleoptiles.

between climatic factors and winter survival, the FROSTOL model makes it possible to perform risk calculations for winter survival of winter cereals under different climatic conditions and to evaluate consequences of anticipated future climatic changes for overwintering. Increased weather variability with repeated cycles of freezing and thawing is expected to be one of the consequences of climate change. RYE-SUS will advance current models (Mirschel *et al.* 2005, Balkovič *et al.* 2013) to reproduce the whole phenology process between sowing and maturity for rye by (i) parametrizing the genotype of semi-dwarf and standard rye hybrids and (ii) integrating their ability to tolerate dehardening and rehardening conditions during winter and hence their robustness in face of climate change. As such, RYE-SUS will integrate studies of different rye genotypes ability to tolerate dehardening and rehardening conditions during winter and hence their robustness in face of climate change.

Conclusions

On a global scale, rye contributes to crop species diversity mainly from the North German Plain to the Ural Mountains and between the 50th and 60th latitude. Farmers in the Rye Belt are globally in competition with other regions of cereal production. Research in RYE-SUS will promote the international competitiveness of rye growing farmers by developing high-performing and environmentally adapted cultivars. We have recently shown, that the rich genetic diversity of the cross-pollinating rye, together with emerging genomic tools, offer a so far underutilized genetic system to enhance our understanding of complex agronomic traits including grain yield in small grain cereals (Hackauf *et al.* 2017).

The genomics-assisted research in RYE-SUS balances current economic models for plant breeding, which have resulted in major investment and subsequent advances for a limited number of major crops, with little, if any, benefit for minor or orphan crops (Abberton *et al.* 2016). RYE-SUS is going to counterbalance this development by tapping the full yield potential of 'the overlooked

cereal' (Buttriss 2006) rye. The breeding research of RYE-SUS is directed to a sustainable intensification in marginal production environments. RYE-SUS advances the Green Revolution with its achievement in terms of agricultural productivity improvement, as we will utilize heterosis in rye to develop genotypes, which gain an improved yield potential and counterbalance drought stress effects through a genetic mechanism. The output of RYE-SUS will increase the competitiveness of rye in agricultural production systems and will ensure access to the health potential of rye bread and other rye-based foods (Jonsson *et al.* 2018).

The demand-less rye enabled post-Roman societies in Europe to survive, but also shaped them in a way that pioneered unique technological developments in world history (Mitterauer 2009). In view on current international efforts to develop solutions combatting the global climate change, rye again offers viable options to help alleviate hunger of a steadily increasing population.

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References

Abberton M, Batley J, Bentley A, Bryant J, Cai H, *et al.* (2016) Global agricultural intensification during climate change: a role for genomics. *Plant Biotechnol J* 14: 1095-1098. DOI: 10.1111/pbi.12467

Auinger HJ, Schönleben M, Lehermeier C, Schmidt M, Korzun V, *et al.* (2016) Model training across multiple breeding cycles significantly improves genomic prediction accuracy in rye (*Secale cereale* L.). *Theor Appl Genet* 129: 2043-2053 DOI: 10.1007/s00122-016-2756-5

Babben S, Schliephake E, Janitza P, Berner T, Keilwagen J, *et al.* (2018) Association genetics studies on frost tolerance in wheat (*Triticum aestivum* L.) reveal new highly conserved amino acid substitutions in CBF-A3, CBF-A15, VRN3 and PPD1 genes. *BMC Genomics* 19: 409. DOI: 10.1186/s12864-018-4795-6

Båga M, Fowler DB, Chibbar RN (2009) Identification of genomic regions determining the phenological development leading to floral transition in wheat (*Triticum aestivum* L.). *J Exp Bot* 60:3575-3585. DOI: 10.1093/jxb/erp199

Bahrani H, Thoms K, Båga M, Larsen J, Graf R, *et al.* (2019) Preferential accumulation of glycosylated cyanidins in winter-hardy rye (*Secale cereale* L.) genotypes during cold acclimation. *Env Exp Bot* 164: 203-212. DOI: 10.1016/j.envexpbot.2019.05.006

Balkovič J, van der Velde M, Schmid E, Skalský R, Khabarova N, *et al.* (2013) Pan-European crop modelling with EPIC: Implementation, up-scaling and regional crop yield validation. *Agric Syst* 120: 61-75. DOI: 10.1016/j.agsy.2013.05.008

Barth R, Bilz M, Brauner R, Clausen J, Dross M, *et al.* (2004) Agrobiodiversität entwickeln! Handlungsstrategien für eine nachhaltige Tier- und Pflanzenzucht. Institut für ökologische Wirtschaftsforschung, Berlin [available at https://www.ioew.de/publikation/agrobiodiversitaet_entwickeln].

Bauer E, Schmutzer T, Barilar I, Mascher M, Gundlach H, *et al.* (2017) Towards a whole-genome sequence for rye (*Secale cereale* L.). *Plant J* 89: 853-869. DOI: 10.1111/tpj.13436

Bergjord AK, Bonesmo H, Skjelvåg AO (2008) Modelling the course of frost tolerance in winter wheat I: Model development. *Eur J Agron* 28: 321-330. DOI: 10.1016/j.eja.2007.10.002

Bergjord Olsen AK, Persson T, de Wit A, Nkurunziza L, Sindhøj E, Eckersten H (2018) Estimating winter survival of winter wheat by simulations of plant frost tolerance. *J Agron Crop Sci* 204: 62-73. DOI: 10.1111/jac.12238

Blum A (2013) Heterosis, stress, and the environment: a possible road map towards the general improvement of crop yield. *J Exp Bot* 64: 4829-4837 DOI: 10.1093/jxb/ert289

Börner A, Melz G (1988) Response of rye genotypes differing in plant height to exogenous gibberellic acid application. *Arch Züchtungsforsch* 18: 71-74.

Braun EM, Tsvetkova N, Rotter B, Siekmann D, Schwefel K, *et al.* (2019) Gene expression profiling and fine mapping identifies a gibberellin 2-oxidase gene co-segregating with the dominant dwarfing gene *Ddw1* in rye (*Secale cereale* L.). *Front Plant Sci* 10: 857. DOI: 10.3389/fpls.2019.00857

Bundessortenamt (2018) Beschreibende Sortenliste Getreide, Mais, Öl- und Faserpflanzen, Leguminosen, Rüben, Zwischenfrüchte.

Buttriss JL (2006) Rye: The overlooked cereal. *Nutr Bull* 31: 3-5. DOI: 10.1111/j.1467-3010.2006.00539.x

Camut L, Regnault T, Sirlin-Josserand M, Sakvarelidze-Achard L, Carrera E, *et al.* (2019) Root-derived GA12 contributes to temperature-induced shoot growth in Arabidopsis. *Nat Plants* 5: 1216-1221. DOI: 10.1038/s41477-019-0568-8

Chen HI, Li PF, Yang CH (2019) NAC-Like Gene GIBBERELLIN SUPPRESSING FACTOR regulates the gibberellin metabolic pathway in response to cold and drought stresses in Arabidopsis. *Sci Rep* 9: 19226. DOI: 10.1038/s41598-019-55429-8

Clarke CK, Gregory PJ, Lukac M, Burridge AJ, Allen AM, *et al.* (2017) Quantifying rooting at depth in a wheat doubled haploid population with introgression from wild emmer. *Ann Bot* 120: 457-470. DOI: 10.1093/aob/mcx068

Colebrook EH, Thomas SG, Phillips AL, Hedden P (2014) The role of gibberellin signalling in plant responses to abiotic stress. *J Exp Biol* 217: 67-75. DOI: 10.1242/jeb.089938

Dittmer HJ (1937) A quantitative study of the roots and root hairs of a winter rye plant (*Secale cereale*). *Am J Bot* 24: 417-420. DOI: 10.2307/2436424

Dreccer MF, Fainges J, Whish J, Ogonnaya FC, Sadras FO (2018) Comparison of sensitive stages of wheat, barley, canola, chickpea and field pea to temperature and water stress across Australia. *Agric For Met* 248: 275-294. DOI: 10.1016/j.agrformet.2017.10.006

Erath W, Bauer E, Fowler DB, Gordillo A, Korzun V, *et al.* (2017) Exploring new alleles for frost tolerance in winter rye. *Theor Appl Genet* 130: 2151-2164. DOI: 10.1007/s00122-017-2948-7

Fowler DB, Limin AE (2004) Interactions among factors regulating phenological development and acclimation rate determine low temperature tolerance in wheat. *Ann Bot* 94: 717-724. DOI: 10.1093/aob/mch196

Francia E, Rizza F, Cattivelli L, Stanca AM, Galiba G, *et al.* (2004) Two loci on chromosome 5H determine low temperature tolerance in a 'Nure' (winter) × 'Tremois' (spring) barley map. *Theor Appl Genet* 108: 670-680. DOI: 10.1007/s00122-003-1468-9

- Geiger HH, Miedaner T (2009) Rye breeding. In: Carena MJ (Ed), Handbook of plant breeding, Vol. 3: Cereals, pp 151-187. Springer, New York. DOI: 10.1007/978-0-387-72297-9
- George N, Kaffka S (2017) Canola as a new crop for California: A simulation study. *Agron J* 109: 496-509. DOI: 10.2134/agronj2016.04.0247
- Gérardeaux E, Loison R, Palai O, Sultan B (2018) Adaptation strategies to climate change using cotton (*Gossypium hirsutum* L.) ideotypes in rainfed tropical cropping systems in Sub-Saharan Africa. A modeling approach. *Field Crop Res* 226: 38-47. DOI: 10.1016/j.fcr.2018.07.007
- Gouache D, Bogard M, Pegard M, Thepot S, Garcia C, et al. (2017) Bridging the gap between ideotype and genotype: Challenges and prospects for modelling as exemplified by the case of adapting wheat (*Triticum aestivum* L.) phenology to climate change in France. *Field Crop Res* 202: 108-121. DOI: 10.1016/j.fcr.2015.12.012
- Hackauf B, Korzun V, Wortmann H, Wilde P, Wehling P (2012) Development of conserved ortholog set markers linked to the restorer gene *Rfp1* in rye. *Mol Breed* 30: 1507-1518. DOI: 10.1007/s11032-012-9736-5
- Hackauf B, Bauer E, Korzun V, Miedaner T (2017) Fine mapping of the restorer gene *Rfp3* from an Iranian primitive rye (*Secale cereale* L.). *Theor Appl Genet* 130: 1179-1189. DOI: 10.1007/s00122-017-2879-3
- Hackauf B, Haffke S, Fromme FJ, Roux SR, Kusterer B, et al. (2017) QTL mapping and comparative genome analysis of agronomic traits including grain yield in winter rye. *Theor Appl Genet* 130: 1801-1817. DOI: 10.1007/s00122-017-2926-0
- Hackauf B, Siekmann D, Fromme FJ (2018) Hybrid breeding in cereals: lessons from rye. *Proc 68th Plant Breeders Conf*, 20-22 Nov 2017, Raumberg-Gumpenstein, Austria, pp 17-18.
- Hedden P (2003) The genes of the Green Revolution. *Trend Genet* 19: 5-9. DOI: 10.1016/s0168-9525(02)00009-4
- Hepting L (1978) Analyse eines 7x7-Sortendiallels zur Ermittlung geeigneten Ausgangsmaterials für die Hybridzüchtung bei Roggen. *Z Pflanzenzüchtg* 80: 188-197.
- Hübner M, Wilde P, Schmiedchen B, Dopierala P, Gowda M, et al. (2013) Hybrid rye performance under natural drought stress in Europe. *Theor Appl Genet* 126: 475-482. DOI: 10.1007/s00122-012-1994-4
- Hwang C, Correll MJ, Gezan SA, Zhang L, Bhakta MS, et al. (2017) Next generation crop models: A modular approach to model early vegetative and reproductive development of the common bean (*Phaseolus vulgaris* L.). *Agric Syst* 155: 225-239. DOI: 10.1016/j.agsy.2016.10.010
- Jones JW, Hoogenboom G, Porter CH, Boote KJ, Batchelor WD, et al. (2003) The DSSAT cropping system model. *Eur J Agron* 18: 235-265. DOI: 10.1016/S1161-0301(02)00107-7
- Jonsson K, Andersson R, Bach Knudsen KE, Hallmans G, Hanhineva K, et al. (2018) Rye and health - Where do we stand and where do we go? *Trend Food Sci Technol* 79: 78-87. DOI: 10.1016/j.tifs.2018.06.018
- Kobyljanski VD (1972) On the genetics of the dominant factor of short-stawed rye. *Genetika* 8: 12-17.
- Kobyljanski VD, Babuzhina DI (2007) Photosynthesis of different plant organs in short stem rye. *Votr Pflanzenzüchtg* 71: 62-65.
- Kottmann L, Schittenhelm S, Wittich KP, Wilde P (2013) Suitability of canopy temperature depression in a temperate climate with drought-stressed winter rye, determined with three infrared measurement devices. *J Agron Crop Sci* 199: 385-394. DOI: 10.1111/jac.12035
- Kottmann L, Wilde P, Schittenhelm S (2016) How do timing, duration, and intensity of drought stress affect the agronomic performance of winter rye? *Eur J Agron* 75: 25-32. DOI: 10.1016/j.eja.2015.12.010
- Laidig F, Piepho HP, Rentel D, Drobek T, Meyer U, Huesken A (2017) Breeding progress, variation, and correlation of grain and quality traits in winter rye hybrid and population varieties and national on-farm progress in Germany over 26 years. *Theor Appl Genet* 130: 981-998. DOI: 10.1007/s00122-017-2865-9
- Li Y, Böck A, Haseneyer G, Korzun V, Wilde P, et al. (2011) Association analysis of frost tolerance in rye using candidate genes and phenotypic data from controlled, semi-controlled, and field phenotyping platforms. *BMC Plant Biol* 11: 146. DOI: 10.1186/1471-2229-11-146
- Lo SF, Ho TD, Liu YL, Jiang MJ, Hsieh KT, et al. (2017) Ectopic expression of specific GA2 oxidase mutants promotes yield and stress tolerance in rice. *Plant Biotechnol J* 15: 850-864. DOI: 10.1111/pbi.12681
- McLeod G, Gan YT, Payne JF (2000) AC Remington winter rye. *Can J Plant Sci* 80: 605-607. DOI: 10.4141/P99-158
- Melz G (1989) Beiträge zur Genetik des Roggens (*Secale cereale* L.). PhD thesis, Berlin.
- Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc Natl Acad Sci* 88: 9828-9832. DOI: 10.1073/pnas.88.21.9828
- Miedaner T, Müller BU, Piepho HP, Falke KC (2011) Genetic architecture of plant height in winter rye introgression libraries. *Plant Breed* 130: 209-216. DOI: 10.1111/j.1439-0523.2010.01823.x
- Miedaner T, Hübner M, Korzun V, Schmiedchen B, Bauer E, et al. (2012) Genetic architecture of complex agronomic traits examined in two test-cross populations of rye (*Secale cereale* L.). *BMC Genomics* 13: 706. DOI: 10.1186/1471-2164-13-706
- Miedaner T, Haffke S, Siekmann D, Fromme FJ, Roux SR, Hackauf B (2018) Dynamic quantitative trait loci (QTL) for plant height predict biomass yield in hybrid rye (*Secale cereale* L.). *Biomass Bioenergy* 115: 10-18. DOI: 10.1016/j.biombioe.2018.04.001
- Miedaner T, Geiger HH (2015) Biology, genetics, and management of ergot (*Claviceps* spp.) in rye, sorghum, and pearl millet. *Toxins* 7: 659-678. DOI: 10.3390/toxins7030659
- Miedaner T, Herter CP, Goßlau H, Wilde P, Hackauf B (2017) Correlated effects of exotic pollen-fertility restorer genes on agronomic and quality traits of hybrid rye. *Plant Breed* 136: 224-229. DOI: 10.1111/pbr.12456
- Mirschel W, Wenkel K-O, Schultz A, Pommerening J, Verch G (2005) Dynamic phenological model for winter rye and winter barley. *Eur J Agron* 23: 123-135. DOI: 10.1016/j.eja.2004.10.002
- Mitterauer M (2009) Warum Europa? Mittelalterliche Grundlagen eines Sonderwegs. Verlag C.H. Beck, München.
- Munz S, Feike T, Chen Q, Claupein W, Graeff-Hönninger S (2014) Understanding interactions between cropping pattern, maize cultivar and the local environment in strip-intercropping systems. *Agric For Met* 195-196: 152-164. DOI: 10.1016/j.agrformet.2014.05.009
- Paponov IA, Lebedinskai S, Koshkin EI (1999) Growth analysis of solution culture-grown winter rye, wheat and triticale at different relative rates of nitrogen supply. *Ann Bot* 84: 467-473. DOI: 10.1006/anbo.1999.0935
- Petroni K, Pilu R, Tonelli C (2014) Anthocyanins in corn: a wealth of genes for human health. *Planta* 240: 901-911. DOI: 10.1007/s00425-014-2131-1
- Philippot L, Hallin S (2011) Towards food, feed and energy crops mitigating climate change. *Trend Plant Sci* 16: 476-480. DOI: 10.1016/j.tplants.2011.05.007
- Plaza-Wüthrich S, Blösch R, Rindisbacher A, Cannarozzi G, Tadele Z (2016) Gibberellin deficiency confers both lodging and drought tolerance in small cereals. *Front Plant Sci* 7: 643. DOI: 10.3389/fpls.2016.00643
- Qin X, Wang H, He Y, Li Y, Li Z, et al. (2018) Simulated adaptation strategies for spring wheat to climate change in a northern high latitude environment by DAYCENT model. *Eur J Agron* 95: 45-56. DOI: 10.1016/j.eja.2017.12.005

- Rabanus-Wallace MT, Hackauf B, Mascher M, Lux T, Wicker T, *et al.* (2019) Chromosome-scale genome assembly provides insights into rye biology, evolution, and agronomic potential. *bioRxiv*, preprint DOI: 10.1101/2019.12.11.869693
- Ryser P (2006) The mysterious root length. *Plant Soil* 286: 1-6. DOI: 10.1007/s11104-006-9096-1
- Sawamoto T, Nakajima Y, Kasuya M, Tsuruta H, Yagi K (2005) Evaluation of emission factors for indirect N₂O emission due to nitrogen leaching in agro-ecosystems. *Geophys Res Lett* 32: L03403. DOI: 10.1029/2004GL021625
- Schittenhelm S, Kraft M, Wittich KP (2015) Performance of winter cereals on field-stored soil moisture only. *Eur J Agron* 52: 247-258. DOI: 10.1016/j.eja.2013.08.010
- Simkin AJ, López-Calcagno PE, Raines CA (2019). Feeding the world: improving photosynthetic efficiency for sustainable crop production. *J Exp Bot* 70: 1119-1140. DOI: 10.1093/jxb/ery445
- Shi MZ, Xie DY (2014) Biosynthesis and metabolic engineering of anthocyanins in *Arabidopsis thaliana*. *Recent Patent Biotechnol* 8: 47-60. DOI: 10.2174/1872208307666131218123538
- Shull GH (1908) The composition of a field of maize. *Am Breed Ass Rep* 4: 296-301. DOI: 10.1093/jhered/os-4.1.296
- Song GS, Zhai HL, Peng YG, Zhang L, Wei G, *et al.* (2010) Comparative transcriptional profiling and preliminary study on heterosis mechanism of super-hybrid rice. *Mol Plant* 3: 1012-1025. DOI: 10.1093/mp/ssq046
- Starzycki S (1976) Diseases, pests and physiology of rye. In: Bushuk W (ed), *Rye: Production, chemistry and technology*, pp 27-61. Am Ass Cereal Chem, St. Paul, MN.
- Subira J, Ammar K, Alvaro F, Garcia del Moral LF, Dreisigacker S, Royo C (2016) Changes in durum wheat root and aerial biomass caused by the introduction of the *Rht-B1b* dwarfing allele and their effects on yield formation. *Plant Soil* 403: 291-304. DOI: 10.1007/s11104-015-2781-1
- Thomashow MF (2010) Molecular basis of plant cold acclimation: Insights gained from studying the CBF cold response pathway. *Plant Physiol* 154: 571-577. DOI: 10.1104/pp.110.161794
- Torop AA, Dedyayev VG, Tschaykin VV, Dokuchaev VV (2003) The results of rye breeding in the central-Chernosem region of Russia. *Plant Breed Seed Sci* 47: 69-75.
- Vagujfalvi A, Galiba G, Cattivelli L, Dubcovsky J (2003) The cold-regulated transcriptional activator *Cbf3* is linked to the frost-tolerance locus *Fr-A2* on wheat chromosome 5A. *Mol Gen Genomics* 269: 60-67. DOI: 10.1007/s00438-003-0806-6
- Van Rees H, McClelland T, Hochman Z, Carberry P, Hunt J, *et al.* (2014) Leading farmers in South East Australia have closed the exploitable wheat yield gap: Prospects for further improvement. *Field Crop Res* 164: 1-11. DOI: 10.1016/j.fcr.2014.04.018
- Voss-Fels KP, Qian L, Parra-Londono S, Uptmoor R, Frisch M, *et al.* (2017) Linkage drag constrains the roots of modern wheat. *Plant Cell Env* 40: 717-725. DOI: 10.1111/pce.12888
- Voylokov AV, Lykholay AN, Smirnov VG (2015) Genetic Control of Anthocyanin Coloration in Rye. *Russ J Genet* 5: 262-267. DOI: 10.1134/S207905971503020X
- Waines JG, Ehdaie B (2007) Domestication and crop physiology: Roots of Green-Revolution wheat. *Ann Bot* 100: 991-998. DOI: 10.1093/aob/mcm180
- Wojcik-Gront E, Bloch-Michalik M (2016) Assessment of greenhouse gas emission from life cycle of basic cereals production in Poland. *Zemdirbyste -Agriculture* 103: 259-266. DOI: 10.13080/z-a.2016.103.033
- Yeo IY, Lee S, Sadeghi AM, Beeson PC, Hively WD, *et al.* (2014) Assessing winter cover crop nutrient uptake efficiency using a water quality simulation model. *Hydrol Earth Syst Sci* 18: 5239-5253. DOI: 10.5194/hess-18-5239-2014
- Zhang X, Chen S, Sun H, Wang Y, Shao L (2009) Root size, distribution and soil water depletion as affected by cultivars and environmental factors. *Field Crop Res* 114: 75-83. DOI: 10.1016/j.fcr.2009.07.006
- Zheng J, Wu H, Zhu H, Huang C, Liu C, *et al.* (2019) Determining factors, regulation system, and domestication of anthocyanin biosynthesis in rice leaves. *New Phytol* 223: 705-721. DOI: 10.1111/nph.15807

The CharAccess project: a close look at scarlet runner bean diversity

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The scarlet runner bean (*Phaseolus coccineus* L.) is susceptible to heat stress during flowering and pod development. In hot summers, the plants shed pods and flowers, resulting in massive yield losses. Heat-tolerant varieties are the key to a sustainable long-term strategy in scarlet runner bean cultivation in Austria. Aims of the project CHARACCESS were to describe the genetic and phenotypic diversity of the selected runner bean varieties or accessions, and to identify potential molecular markers to facilitate breeding work with regard to heat tolerance. The tested runner bean accessions were mainly of Austrian origin from the AGES-gene bank in Linz (<https://www.genbank.at/en/ecpgr-phaseolus.html>).

In total 84, accessions were selected based on their origin and on seed characteristics according to UPOV. The samples were compared with ten varieties from Austria and the Netherlands. The variety "Bonela" was used as the reference variety as it is widely cultivated in Styria. For DNA extraction, leaf material from eight individuals of each of the 94 genotypes (accessions and varieties) was collected. Genotyping by sequencing was done by RAD-Seq (Restriction site associated DNA sequencing), and data was evaluated using the evaluation softwares ipyrad and Structure as well as various R-packages.

For phenotyping, 23 accessions from the AGES-gene bank and ten varieties were selected with the aim to achieve a maximum of genetic diversity. The ten reference varieties were 'Bonela' (standard) and 'Melange', both registered in the Austrian List of Varieties, old varieties ('Steirische Riesen', 'Hara', 'Choco', 'Hellviolette', 'Aldrian'), and the varieties 'Lady Di', 'Hestia' and 'Prijswinner', registered in The Netherlands. The runner beans were cultivated in the greenhouse and exposed to a daily four-hour heat stress of 35°C over a period of four weeks. Heat stress was applied when half of all assessed individuals were flowering. The pollination of the flowers that is necessary for pod formation was ensured by the presence of honey bee colonies. Selected phenotypic characteristics (number of flowers, number of pods) were assessed over the entire heat stress period.

The first flowers were observed about one month after sowing, and the plants continuously formed new flowers. The honey bees situated in the greenhouse visited the flowers and ensured the pollination of the runner beans. About eight weeks after sowing, the first pods were observed. Pods were formed by 32 out of 33 accessions and varieties, with more than half of the plants showing pods larger than 5 cm. On average, it took 14 days from the first flower observed on a plant to the first pod being formed. As expected, all 33 accessions and varieties responded to the heat stress by shedding flowers and pods. The total number of flowers for all 33 accessions and varieties decreased from 1884 flowers to 327 flowers within one week after onset of the heat stress. Already well-developed pods were usually not shed by the plants. No new pods and no more flowers were formed during the forced heat stress.

Barely two weeks after the end of the heat stress, the honey bees were again observed on the flowers, resulting in the formation of new pods a few days later. From nine accessions, as well as from the old variety 'Hara', more beans were harvested than from the reference variety 'Bonela'. One runner bean accession from Italy and one from Burgenland clearly stood out as the highest-yielding accessions. This was followed by a large upper midfield, which included accessions mainly from Styria and Burgenland as well as the variety 'Hara'. The remaining 22 runner bean accessions and varieties were all less productive than the reference variety.

The analysis of the structure of the genetic relationship between the accessions showed that one accession, originating from China, was clearly distinct from the other genotypes. Two large groups could be defined, whose accessions or varieties are more closely related within the group. The variety 'Bonela' is found in one of these groups.

In further studies, accessions can be chosen that are genetically similar to those accessions described here as heat-tolerant. In the phenotype-genotype association analysis the correlation of 483

loci from 33 genotypes with six expressions was tested. In total, 28 associated genomic regions, that are potentially associated with heat tolerance were found and are the basis for further steps towards the development of genetic markers associated with a particular trait. Further genotyping and phenotyping work on additional accessions is necessary to verify whether the genomic regions identified are indeed associated with the phenotypic expression of heat tolerance. The final result will be molecular markers that can be used in further breeding and selection processes. The project CHARACCESS provided a solid basis for further breeding work regarding heat tolerance of the scarlet runner bean.

Keywords

heat tolerance · molecular marker · *Phaseolus coccineus* · plant genetic resources · yield

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Harvesting the potential: guided domestication of perennial bioenergy crops

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Humans have always used crops for energy production. To achieve carbon-neutral energy production, multiple legal directives pushed to increase the cultivation of 1st generation energy crops in the European agricultural landscape to grow the biomass needed for big-scale biogas or pellet production. Hence, the cultivation of annual bioenergy crops in highly managed agro-ecosystems has greatly increased in the last couple of decades. This domination of annual energy crops in a highly intensive agricultural system, with corn (*Zea mays*) as the primary example, has been noted to cause multiple environmental problems like soil erosion, high use of pesticides and herbicides, reduced biodiversity, the spread of new pests and diseases as well as a reduction of food sources for pollinating insects.

The two undomesticated, perennial bioenergy crops *Silphium perfoliatum* and *Sida hermaphrodita* both possess defined traits, which are associated with an additional value for its respective ecosystem. These include a long flowering period, which can serve as a food source for pollinating insects in an otherwise sparse agricultural landscape as well as reduced erosion of the soil due to the permanent soil cover, deep roots and the lack of necessity for yearly tillage that usually accompanies annual crop production. Additionally, the crops' extensive root system increases carbon sequestration and nutrient uptake efficiency, potentially allowing both *S. perfoliatum* and *S. hermaphrodita* to be grown in a low-input system on marginal land. Target-oriented breeding could

further raise the potential of both non-domesticated perennials. Using a structured approach guided by an ideotype in mind can increase the agronomic potential, while simultaneously preserving wild traits with valuable ecosystem functions.

Today, the agricultural landscape is dominated by annual crops with an annual harvest as a return on investment, which are a result of a domestication process millennia ago that had to guarantee the year-to-year survival of the human race. This domestication process was propelled by an annual selection for yield and defect elimination, thereby limiting the crops in their environmental function as these traits were not sought after. As a result, most modern annual crops provide minimal ecosystem services. In modern times, with food supply being abundant in the Western world, humanity has the luxury to look at a crop and the role it could potentially fulfil in an agricultural ecosystem, including all its possible ecosystem services. This idea is called an ideotype and is defined as a planned and theoretically constructed phenotype whose behavior and performance in a defined environment can be predicted and expected. Having a defined ideotype in mind gives breeders and crop breeding programs a strategy and an end goal for genetic selection. Since domesticated crops are very limited in their agricultural function, they form an unsuitable basis for the development of ideotypes, which would be high in ecosystem services. Hence, non-domesticated crops with high genetic variation and diversity should be used as a starting point to breed to-



Figure 1: Inflorescences of *Silphium perfoliatum* (left) and *Sida hermaphrodita* (right). Both crops are rich sources of nectar and pollen for honeybees.



Figure 2: Two-year old *Sida hermaphrodita* stand during the summer months.

wards a previously defined crop ideotype with a maximum of ecosystem services. Due to the defined traits mentioned above, which are associated with an additional value for the crops' respective ecosystem, a proposition could be made to use both *S. perfoliatum* and *S. hermaphrodita* as a base model to develop new bioenergy crops ideotypes.

A proposed perennial ideotype for a guided domestication process based on the non-domesticated crops *S. perfoliatum* and *S. hermaphrodita* would, for one and foremost, keep the perennial nature of the crops. Additionally, the crops prominent inflorescence which serve as a pollinator food source should be kept during the domestication process, while simultaneously breeding for low seed dormancy and a synchronized germination which helps with stand establishment by sowing. Yield could be increased by selecting towards an ideotype with bigger shoot diameter, higher plant height and a greater internode number, while lignin content should be increased/decreased in accordance with the crops' energetic utilization strategie, i.e. either biogas production or thermic utilization of the lignified biomass. Additionally, it is important to preserve any resistance to diseases found in the undomesticated perennials. If all these beneficial traits are being considered during the guided domestication, the result would be a promising bioenergy crop with a high value for its respective agricultural ecosystem.

Keywords

Biogas · breeding · ideotype · *Sida hermaphrodita* · *Silphium perfoliatum*

References

- Donald CT (1968) The breeding of crop ideotypes. *Euphytica* 17: 385-403. DOI: 10.1007/BF00056241
- Gansberger M, Montgomery LFR, Liebhard P (2015) Botanical characteristics, crop management and potential of *Silphium perfoliatum* L. as a renewable resource for biogas production: a review. *Ind Crop Prod* 63: 362-372. DOI: 10.1016/j.indcrop.2014.09.047
- Nahm M, Morhart C (2018) Virginia mallow (*Sida hermaphrodita* (L.) Rusby) as perennial multipurpose crop: biomass yields, energetic valorization, utilization potentials, and management perspectives. *GCB Bioenergy* 10: 393-404. DOI: 10.1111/gcbb.12501
- Van Tassel DL, Albrecht KA, Bever JD, Boe AA, Brandvain Y, *et al.* (2017) Accelerating *Silphium* domestication: an opportunity to develop new crop ideotypes and breeding strategies informed by multiple disciplines. *Crop Sci* 57: 1274-1284. DOI: 10.2135/cropsci2016.10.0834