



# 71<sup>st</sup> Conference

23-24 November 2020  
Online Conference

Breeding for resilience against biotic and abiotic stresses



Vereinigung der Pflanzenzüchter  
und Saatgutkaufleute Österreichs

# 71. Tagung

23.-24. November 2020

Online Conference

Breeding for resilience against biotic and abiotic stresses

**SAATGUT**  
AUSTRIA 



## **Impressum**

**Tagungsband der 71. Jahrestagung der Vereinigung  
der Pflanzenzüchter und Saatgutkaufleute Österreichs,  
23.-24. November 2020, Online Conference**

### ***Herausgeber***

Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs  
Wiener Str. 64  
3100 St. Pölten  
Email: [office@saatgut-austria.at](mailto:office@saatgut-austria.at)  
URL: [www.saatgut-austria.at](http://www.saatgut-austria.at)

### ***Redaktion***

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A.o. Univ.Prof. Dr. Heinrich Grausgruber  
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### ***Für den Inhalt verantwortlich***

die Autoren

### ***Foto Titelblatt***

Rizky Psthika Kirana, Universität für Bodenkultur Wien, Campus Tulln

### ***Druck und Verlag***

Department für Nutzpflanzenwissenschaften  
Universität für Bodenkultur Wien  
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3430 Tulln an der Donau  
Email: [pflanzenzuechtung@boku.ac.at](mailto:pflanzenzuechtung@boku.ac.at)

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ISBN-13: 978-3-900932-81-7

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## Obituary: Konrad Schulmeister (1933-2019)

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Our breeder colleague Konrad Schulmeister died on December 18, 2019 at the age of 86 years.

Konrad Schulmeister was born on November 15, 1933 as a farmer's son in Blumau near Etzen in the Austrian Waldviertel. During his education at the Edelhof Agricultural College near Zwettl he had contact with plant breeding for the first time. After finishing school he was employed as a technician by the former head of the Edelhof breeding programme, Johann Boden, later also director of the school. From 1954 onwards, Schulmeister worked as assistant in cereal breeding and as teacher in the agricultural college at Edelhof. From 1979 until his retirement in 1997, Schulmeister was the head of plant breeding at Saatzeit Edelhof. Even in retirement he was obliged to breeding until 2009 by his engagement in the „Verein zur Förderung der Mohn- und Getreidezüchtung“, an association for the promotion of poppy and cereal breeding. At the age of 76 he left „his Edelhof“ with a heavy heart, satisfied to know that the continuation of breeding was in good hands.

Schulmeister started in 1954, with modest means and by self-learning, selection in old rye landraces. With a persistence characteristic to the residents of the Waldviertel, he developed today's Edelhof plant breeding programmes in an impressive manner. After winter rye and oat, breeding programmes were initiated in 1973 for spring barley, in 1977 for wheat and in 1990 for winter barley. Schulmeister also included grey poppy as a speciality of the Waldviertel region in the breeding work.

Schulmeister also intensified the cooperation with the VLG, the Association of Rural Cooperatives (today RWA - Raiffeisen Ware Austria) and with numerous foreign companies as seed and variety distribution partners. In order to save time in breeding, winter generations in Chile were established in 1993. In the same year, the Association for the Promotion of Poppy and Cereal Breeding was founded in Zwettl. Under Schulmeister as managing director and later as chairman, the association developed into an important project sponsor for investment, research and development projects. In total, 16 R&D projects were financed under the responsibility of national and EU funding agencies. The topics ranged from "Population rye with special winter hardiness for Austrian mountainous regions" or "Bioethanol - energy from grain" to his most beloved project "2/3 seed demand - 2/3 breeding time in winter wheat".



With the inauguration in 1996, Schulmeister was able to fulfill his desire of more than two decades, namely to manage an own house for the breeding activities at Edelhof. Since then, a stylish three-sided Waldviertel homestead has been used by Saatzeit Edelhof and the Association for the Promotion of Poppy and Cereal Breeding. The title "Ökonomierat" awarded in 1998 by the Ministry of Agriculture was a great acknowledgement for Konrad Schulmeister and his breeding activities. He has made a significant contribution to the development of Austrian agriculture.

Under the coordination of Konrad Schulmeister in cooperation with RWA, the 100<sup>th</sup> anniversary of the cereal breeding at Edelhof was celebrated in 2003. Furthermore Mr. Schulmeister had been a member of the steering committee of Saatgut Austria, the Association of Austrian Plant Breeders and Seed Traders, for many years.

Many variety registrations of winter rye, spring oats, spring barley, spring and winter wheat, and grey poppy are the achievement of Konrad Schulmeister, in Austria as well as in many European countries: (i) winter rye: EHO-Kurz (1965; AT, CH, FR, HR), Elect (1992; AT, CH, SI); (ii) spring oats: Edo (1987; AT, CH), Eberhard (1989; AT, CH, HU, LU), Explorer (1989; AT, HR, LU), Euro (1991;

AT, SI, SK), Expander (1992; AT, CH, DE, HR, HU, LU, SI, SK), Efendi (1995; AT, CH), Ewald (1997; AT); (iii) spring barley: Ebra (1984; AT), Elke (1986; AT), Erika (1988; AT), Esta (1989; AT), Extra (1990; AT, HR), Effekta (1991; AT, HR), Elisa (1993; AT, CH, HU, LU, SI), Evelyn (1994; DK); (iv) spring wheat: Erwin (1990; AT), Egon (1995; SI); (v) winter wheat: Exquisit (1995; AT), Erik (1996; HU), Edison (2001; AT, HR); (vi) grey poppy: Edel-Rot (1990; AT), Edel-Weiß (1990; AT). In addition, Schulmeister set the basis for the selection of many more varieties bred at Edelhof. From the early days, when he worked almost alone in the fields, the staff grew to six employees until he retired. At his beginnings in the 1950s bree-

ding at Edelhof was selection for the region. With his dedication, Saatzucht Edelhof became an international well known plant breeding company.

Konrad retired, but had been continually connected to Edelhof as a breeder up to the very end of his life. Our breeding team at Edelhof will all the time keep an honorable memory of its mentor and great role model Konrad Schulmeister. Also the „Gumpenstein community“ and Saatzucht Austria will always remember Konrad Schulmeister.

## Stability of yield and baking quality parameters of heterogeneous wheat populations

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### Abstract

In this study, heterogeneous winter wheat populations are compared to commercial pure line varieties in terms of performance and stability of yield and baking quality parameters. Comparative field trials were conducted in Germany and Switzerland under organic conditions over two years at four sites (trial 1) and over three years at 5-6 sites (trial 2). The test entries include heterogeneous winter wheat populations representing contrasting genetic backgrounds, among them officially registered populations within the temporary experiment 2014/150/EU. As reference varieties commercial cultivars of the highest German baking quality category 'E' suitable for organic production were used. Grain yield, wet gluten content and sedimentation value were measured in both trials and protein content in trial 2. The results of the trials indicate that two of the officially registered populations have a yield potential and baking quality characteristics comparable to the mean of the reference varieties. Other populations reached either lower yield and higher baking quality than the reference varieties or vice-versa. The stability analysis shows that the populations exhibited a distinctly higher dynamic as well as static stability for all baking quality parameters compared to the varieties. Averaged over all three quality parameters, the mean values of the stability measures for populations were 55% (dynamic) and 27% (static) lower than the means of the varieties. Notably, the two officially registered populations ranked first and second among the test entries for static stability for all quality parameters. The yield stability analysis indicated a tendency towards a higher dynamic stability in the populations.

### Keywords

Organic farming · protein content · sedimentation value · *Triticum aestivum* · yield trial

### Introduction

Increasingly extreme environmental stresses caused by climate change will severely impact future agricultural production (Mbow *et al.* 2019). Breeding strategies with the aim to stabilize yield as well as quality traits of crops across variable environments are therefore urgently required. An additional approach to multi-environment testing (MET) or resistance breeding may be to utilize genetic diversity directly in the field in the form of heterogeneous cereal populations (Soliman & Allard 1991). Heterogeneous populations are resulting from genetically diverse parental germplasm, being managed as bulk populations with or without conscious selection by breeders. A legal framework for the marketing of heterogeneous populations will be provided by the new organic regulation, which will come into force on January 1, 2022.

There are „static“ as well as „dynamic“ concepts of stability (Becker & Léon 1988). A statically stable genotype tends to maintain the same performance across environments (*i.e.* location by year combinations). A dynamically stable genotype performs parallel to the mean of each environment thus corresponding to low genotype×environment interactions. Dynamic stability measures have the advantage that they reward genotypes that show a positive response to favorable environmental conditions. On the other hand, the static stability concept has a more straightforward agronomic interpretation since it measures the security of agricultural production (Annicchiarico 2002). Although the two concepts account for different aspects of stability, certain wheat genotypes can be superior to others for both stability measures (see *e.g.* Knapp *et al.* 2017).

The static and dynamic stability of heterogeneous cereal populations was investigated in prior studies: Döring *et al.* (2015) found that wheat composite cross populations tended to exhibit a grea-

ter static stability of grain and protein yield compared to the mean of their parental varieties. Studying the same populations over several generations in Germany, Weedon & Finckh (2019) demonstrated that the populations had a tendency towards a higher dynamic grain yield stability than commercial pure line varieties under organic management. However, no difference was found for static stability measures. In Italian trials conducted by Raggi *et al.* (2017), heterogeneous spring barley populations achieved a higher level of dynamic yield stability than the pure line reference varieties, but a similar stability compared to pure lines derived from the populations.

It should be noted that all of these studies also investigated other aspects of heterogeneous populations and were not exclusively designed as yield stability assessments. The aim of our analysis is to extend the experimental setup in two respects: First, the number of test environments should be increased. The above mentioned studies were carried out in 8 to 12 environments. The minimal number of test environments required for stability assessments is considered eight according to Kang (1998) and Piepho (1998). Second, the heterogeneous populations should include populations with contrasting genetic background and in particular, a similar number of populations and pure line reference varieties should be tested in the analysis.

## Material and methods

### Plant material and field trials

The datasets evaluated in this study originate from two multi-environment winter wheat field trials under organic management. The first trial included twelve heterogeneous populations (among them the officially registered ‘Evolito A - E’, ‘Brandex’ and ‘Liocharls’ populations within the temporary experiment 2014/150/EU of the European Commission) and five pure line reference varieties of the baking quality E (‘Aristaro’, ‘Wiwa’, ‘Butaro’, ‘Trebelir’, ‘Genius’). The trial was conducted as randomised complete block design with four replications at four locations (Dottenfelderhof, DE, plot size: 9 m<sup>2</sup>; Gladbacherhof, DE, 9 m<sup>2</sup>; Forchheim am Kaiserstuhl, DE; 12.8 m<sup>2</sup>; Feldbach, CH, 4 m<sup>2</sup>, 3

replications) in two years (2019 and 2020). The second trial (Ökolandessortenversuche Baden-Württemberg - the official organic variety trials of the Federal State of Baden-Württemberg) included six populations (in addition to Brandex and Liocharls the populations OQI and OYQII studied in Weedon & Finckh (2019), as well as the population CC2K from Agroscope/DSP, CH, and CCPWS from TU München) and eight E wheat varieties (‘Alessio’, ‘Aristaro’, ‘Baretta’, ‘Butaro’, ‘Ponticus’, ‘Royal’, ‘Titlis’, ‘Trebelir’). This trial was also conducted as randomised complete block design with four replications at 5 to 6 locations in Baden-Württemberg representing 4 to 5 soil-climate areas (Hohenheim, Crailsheim, Karlsruhe-Grötzingen, Forchheim am Kaiserstuhl, Ochsenhausen, Maßhalderbuch) for three years (2018, 2019 and 2020). The plot size varied between 10.5 and 14.4 m<sup>2</sup> depending on year and location. The field trials were evaluated according to the guidelines of the German Federal Plant Variety Office (Bundessortenamt 2000). Baking quality analysis were conducted for the parameters protein content (%) (only available for trial 2), wet gluten content (%) and sedimentation value (mL) (trial 1: SDS sedimentation test, trial 2: Zeleny sedimentation test).

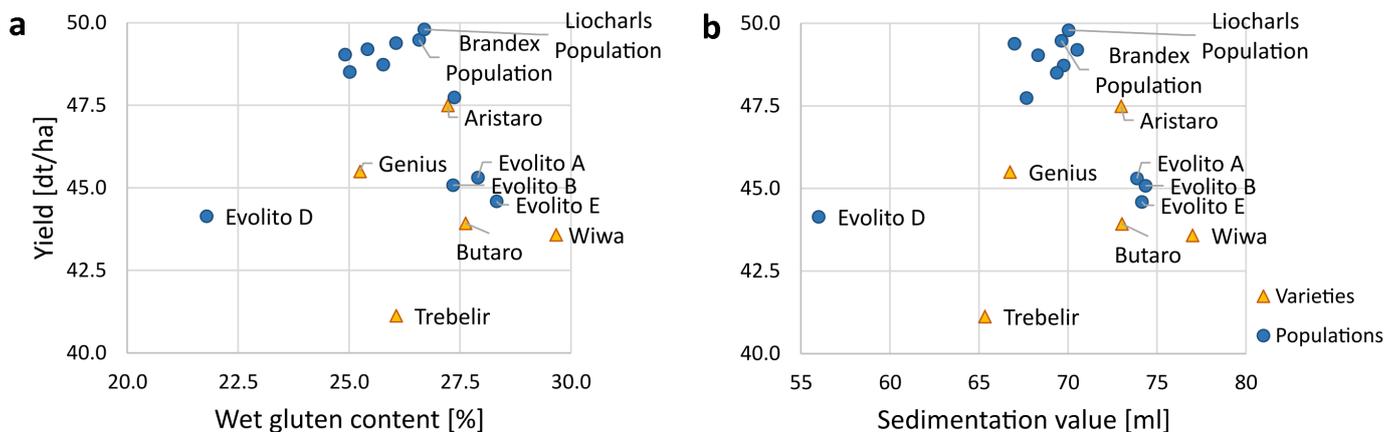
### Stability measures

The results of the stability analysis are only presented for trial 2 with the 16 year×location combinations considered as environments. For the calculation of the dynamic stability of the test entries Wricke’s ecovalence  $W_i^2$  (Wricke 1962) was applied and as a static measure the environmental variance  $EV_i$  (see Becker & Léon (1988) and Annicchiarico (2002) for definitions). The greatest stability is achieved at  $W_i^2 = 0$  and  $EV_i = 0$ , respectively.

## Results and discussion

### Mean yield and baking quality performance

In trial 1 all but one of the twelve populations had higher grain yields than the mean of the varieties. Four populations exhibited higher protein content and three populations exhibited higher sedimentation value than the mean of the check varieties (Figure 1). In trial 2 there was no difference in grain yield between the mean of the populations and the mean of the varieties. The means



**Figure 1** Relationship between grain yield and quality parameters of check varieties and populations of winter wheat in trial 1 ( $n=8$ ): **a** wet gluten content; **b** SDS sedimentation value. Values represent adjusted means across environments; triangles indicate pure-line varieties, circles indicate populations.

**Table 1** Mean value, environmental variance  $EV_i$  and Wricke's ecovalence  $W_i^2$  for grain yield (at 14% H<sub>2</sub>O), protein content, wet gluten content and Zeleny sedimentation value for check varieties and populations of trial 2 ( $n=16$ ).

Entry	Grain yield (dt/ha)			Protein content (%)			Wet gluten content (%)			Zeleny sedimentation value (mL)		
	Mean	$EV_i$	$W_i^2$	Mean	$EV_i$	$W_i^2$	Mean	$EV_i$	$W_i^2$	Mean	$EV_i$	$W_i^2$
Check variety												
Alessio	50.8	132.0	60.4	12.2	0.8	1.7	26.9	3.8	14.5	44.5	41.3	162.7
Aristaro	49.9	96.3	68.9	11.8	0.7	4.0	26.2	5.4	48.1	39.0	61.6	486.0
Baretta	52.8	149.3	148.8	11.1	0.8	1.5	24.2	4.0	8.6	34.7	56.5	311.2
Butaro	45.8	88.0	159.6	12.1	1.0	1.3	26.3	4.4	13.5	42.3	46.2	111.7
Ponticus	52.4	140.5	139.0	10.9	0.9	1.2	23.5	4.4	8.3	32.6	51.9	145.9
Royal	52.3	141.1	108.2	11.3	1.1	3.1	24.1	5.8	30.1	38.9	67.3	437.9
Titlis	50.3	102.7	105.3	11.8	1.4	5.0	26.1	5.7	16.6	38.8	84.8	351.2
Trebelir	47.4	113.8	69.4	11.9	0.8	2.3	25.9	4.3	15.0	37.6	58.8	350.3
Mean	50.2	120.5	107.4	11.6	0.9	2.5	25.4	4.7	19.3	38.5	58.5	294.6
Population												
Brandex	50.2	126.5	117.0	11.6	0.5	0.7	25.4	2.5	5.6	38.4	30.1	44.4
Liocharls	50.3	125.9	51.1	11.8	0.6	0.5	25.7	3.5	12.5	39.0	35.5	50.8
CCPWS	50.3	128.8	57.3	11.5	0.7	1.2	24.7	4.2	15.8	35.5	46.0	111.0
CC2K	50.6	91.8	66.6	11.4	0.8	0.9	24.7	4.0	7.7	34.6	50.0	125.9
OQI	49.7	105.1	27.8	11.5	0.7	1.4	25.0	3.9	14.0	35.8	46.0	133.9
OYQII	51.4	112.7	107.5	11.0	0.6	0.6	23.5	3.9	7.2	30.8	53.8	215.5
Mean	50.4	115.1	71.2	11.5	0.7	0.9	24.8	3.7	10.5	35.7	43.6	113.6

of the protein and wet gluten content of the populations were slightly lower than the means of the varieties (11.5% vs. 11.6% and 24.8% vs. 25.4%). The mean sedimentation value of the populations was 7% lower compared to the mean of the varieties (35.7 mL vs. 38.5 mL). In trial 2 'Brandex' and 'Liocharls' reached mean grain yields and mean baking quality values almost exactly equal to the mean of the varieties for all parameters (Table 1).

#### Yield stability

Most likely due to the limited number of test environments, conclusive results could not be obtained from the stability analysis of trial 1. The populations of trial 2, however, showed a tendency towards higher dynamic grain yield stability compared to the pure line reference varieties which is in line with the findings of Weedon & Finckh (2019) for wheat and Raggi *et al.* (2017) for barley. The mean  $W_i^2$  value was 34% lower for the populations compared to the check varieties, indicating a higher dynamic yield stability of the populations. Still, four of the varieties had lower  $W_i^2$  values than two of the populations (Table 1). The static stability analysis did not reveal clear differences between populations and varieties since the mean  $EV_i$  value between the two groups differed only slightly (4% lower for the populations).

#### Stability of baking quality parameters

For the baking quality parameters, all populations achieved greater dynamic as well as static stability than the mean of the varieties. The mean  $W_i^2$  value of the populations was 64% (protein content), 46% (wet gluten content) and 61% (sedimentation value) lower compared to the mean of the varieties. The mean  $EV_i$  value of the populations was 28% (protein content), 22% (wet gluten content) and 26% (sedimentation value) lower than the mean of the varieties. Ordering the test entries according to the values of

the stability measures (with lower values first), the populations always occupied the first two ranks and four out of the first six ranks (Table 1).

Notably, the populations 'Brandex' and 'Liocharls' ranked first and second for  $EV_i$  for all three quality parameters. In the case of  $W_i^2$  at least one of the populations always occupied the first rank. In particular, these results confirm that genotypes can have both a higher dynamic as well as static stability relative to other test entries, in line with the findings of Knapp *et al.* (2017).

Taking into account the absolute values of the quality parameters by considering *e.g.* for the static stability the coefficient of variation (CV%) instead of  $EV_i$  does not change the general assertions outlined above. 'Brandex' and 'Liocharls' still rank first and second for the stability of all quality parameters, except for sedimentation value where they rank first and third. Moreover, in all but one case, the CV% of all populations are still below the mean of the varieties.

In conclusion, the results of this study demonstrate that the tested populations exhibited both a higher dynamic and static stability than the pure line reference varieties for the baking quality parameters protein content, wet gluten content and sedimentation value. These findings are well substantiated by the high number of test environments and the representative set of eight reference varieties with excellent baking quality under organic growing conditions. We shall defer the investigation of the statistical significance of the results in this study as well as investigations of reliability indices, which combine the mean performance and stability measure in one parameter, to future work.

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## Preliminary findings from ERA-Net WheatSustain: Knowledge-driven genomic predictions for sustainable disease resistance in wheat

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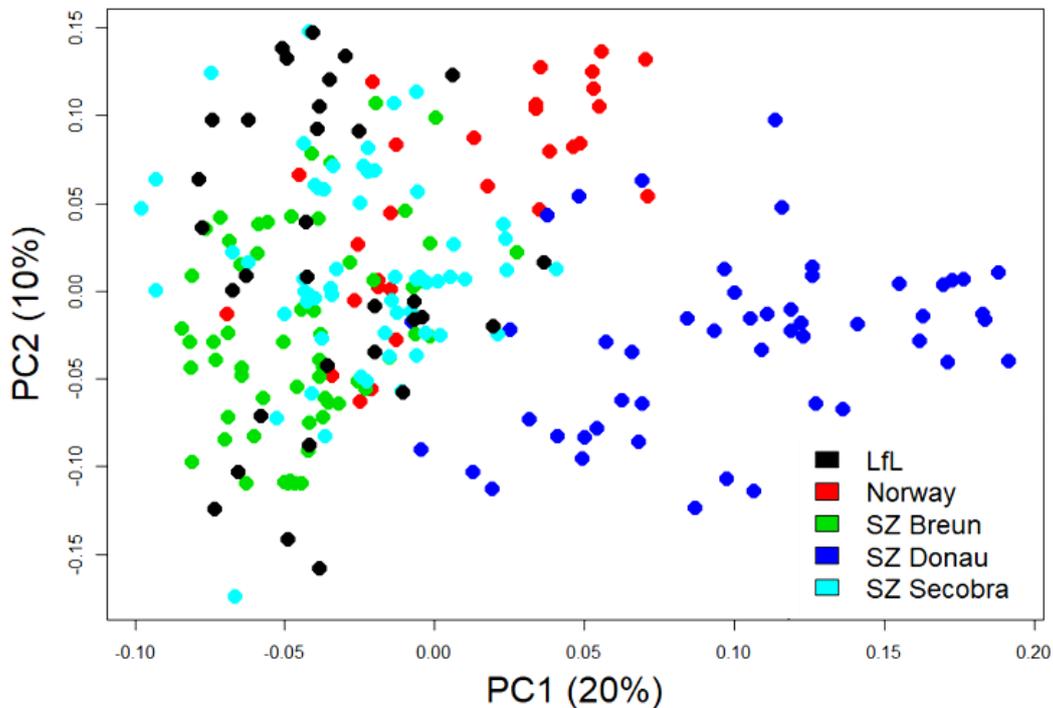
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Here we present preliminary findings from the SusCrop – ERA-NET funded project entitled “WheatSustain: Knowledge-driven genomic predictions for sustainable disease resistance in wheat”. The project consortium comprises partners with expertise in genomic prediction, plant pathology, and plant breeding from public and private institutions in Austria (BOKU, Saatzzucht Donau), Germany (LfL, Secobra Saatzzucht, Saatzzucht Breun), Norway (NMBU, Graminor), Ireland (UC Dublin), Canada (Agriculture and Agri-Food Canada), Mexico (CIMMYT), and USA (StatGen Consulting). *Fusarium* head blight (FHB) and stripe rust (*Puccinia striiformis*), two economically important diseases of wheat with distinct genetic architectures, are used as models in the WheatSustain project. FHB resistance is quantitatively controlled, has significant genotype by environment interactions (G×E), and is correlated with developmental traits, such as flowering time and anther extrusion. Resistance to stripe rust is controlled by both major-effect loci that are non-durable and race-specific and minor-effect loci that are durable but can be masked by major loci. We propose to improve genomic prediction for these traits with methods such as utilizing validated loci, leveraging correlations with high-heritability traits, distinguishing race-specific from non-race-specific loci, and modeling epistasis. The project has six work packages (WP): creating joint phenotypic and genotypic datasets (WP1); comparing alternative genomic prediction strategies by computer simulation (WP2); FHB case study (WP3); stripe rust

case study (WP4); validation of prediction models (WP5); and project management and dissemination (WP6). We present current results from WP1, WP3, and WP4.

The aims of WP1 have largely been met. The training population comprises 230 breeding lines from LfL (N=32), Norway (N=30), Saatzzucht Breun (N=59), Saatzzucht Donau (N=50), and Secobra Saatzzucht (N=59) and was genotyped with a 25K single nucleotide polymorphism (SNP) chip (SGS-TraitGenetics GmbH, Gatersleben, Germany). A principal component analysis of the 25K markers demonstrated that the first two principal components (PCs) explained 30% of the variance in the training population, with the Saatzzucht Donau lines separated from the other material on the first PC (20% variance) and the second PC (10% variance) showing some separation between the Norwegian lines and the German and Austrian lines (Figure 1). The first FHB field trials for WP3 were conducted in 2020 in Ås, Norway (NMBU) and Tulln, Austria (BOKU) with two replications each and in Feldkirchen, Germany (Secobra) and Reichersberg, Austria (Saatzzucht Donau) with one replication each. All trials were artificially inoculated with *Fusarium culmorum* or *F. graminearum* and the plots were phenotyped for FHB severity, days to anthesis (DTA), anther extrusion (AE), and plant height (PH). Across trials, FHB severity was negatively correlated with AE ( $r=-0.11$ ) and positively correlated with DTA ( $r=0.30$ ). AE and PH were positively correlated with each other ( $r=0.21$ ) and negatively with DTA ( $r=-0.27$ ). Broad-sense heritability ( $H^2$ ) was high for all traits ( $H^2_{FHB}=0.97$ ;  $H^2_{AE}=0.66$ ;  $H^2_{DTA}=0.88$ ;  $H^2_{PH}=0.99$ ).



**Figure 1** Principal component analysis of the 25K markers of the WHEATSUSTAIN training population

Genome-wide association (GWA), with control of population structure using a kinship matrix derived from the 25K SNPs, identified 5 SNPs significantly associated with FHB severity in the training population on chromosome 2A at 2.65, 3.11, and 31.60 Mbp, on chromosome 2B at 23.12 Mbp, and on chromosome 4D at 18.78 Mbp. The significant GWA SNPs each explained a small proportion of the variance in FHB ( $R^2=0.04$ , marker effect=-0.5). The significant SNP on chromosome 4D is a marker for the semi-dwarfing gene *Rht-D1* and was also significantly associated in GWA for plant height. For genomic prediction of FHB within the training population, we used cross-validated (5-fold, 10 replicates) genomic best linear unbiased prediction (GBLUP), in which the kinship matrix was modeled as a random effect and the *Rht-D1* SNP was included as a fixed effect. GBLUP with the kinship matrix alone had a predictive ability of  $0.70 \pm 0.03$  and GBLUP with the kinship matrix and the *Rht-D1* marker had a slightly higher predictive ability of  $0.71 \pm 0.03$ . For WP4, stripe rust field trials were planned in 2020 in Herzogenaurach (Saatzucht Breun) and Lemgo (Secobra Saatzucht) in Germany and in Reichersberg (Saatzucht Donau) and Tulln (BOKU) in Austria with one replication each. Although all trials were artificially inoculated with *P. striiformis*, there was only sufficient disease pressure for stripe rust scoring in Lemgo. Stripe rust severity was scored at three timepoints after inoculation on each plot. Stripe rust severity was uniformly low at the first two time points (most plots <5%). The third timepoint demonstrated wider variation and higher severity than the first two scorings. The first score was moderately correlated with the second ( $r=0.55$ ) and third ( $r=0.28$ ) scores, and the second and third scores were highly correlated ( $r=0.72$ ). We found haplotypic diversity at SNPs associated with two known stripe rust resistance genes, with six haplotypes at *Yr5* and 10 haplotypes at *Yr15*.

#### Keywords

Dwarfing gene · Fusarium head blight · GBLUP · genomic selection · *Puccinia striiformis* · *Triticum aestivum* · wheat breeding

#### Acknowledgements

The project WheatSustain (DaFNE-101402) was carried out under the ERA-NET Cofund SusCrop (Grant N°771134), being part of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI).

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## Genomic prediction of stripe rust resistance in wheat. Opportunities, limitations and lessons learned from a breeders' population

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Bread wheat is one of the most important food crops, providing 20% of calories and protein worldwide. Stripe rust (*Puccinia striiformis* f.sp. *tritici*) is a major threat to wheat production and, especially in temperate climates, causing up to 40% losses in yield. Recent epidemics, caused by newly evolved pathogen races are rendering breeding efforts void and demand novel solutions. Resistance breeding is the most effective way of counteracting stripe rust. It relies on phenotypic and marker-assisted selection (MAS) to combine resistance genes in high yielding genotypes. Phenotypic selection is however, only possible in years with high disease pressure. MAS relies on a few or even single markers, that are associated with resistance genes which can easily be overcome by pathogens. New genotyping methods and improved statistical methods enable prediction and selection based on a large number of markers, ultimately enabling genomic prediction of traits. Recent studies have shown that genomic prediction can be used to predict disease resistance in wheat, however more research is needed on the durability of predictions when races are changing. The goal of this study was therefore to find out if genomic prediction can be used to improve quantitative, non-race-specific resistance against stripe rust in wheat.

Data from wheat breeding trials in the years 2013 to 2016, as well as genotyping by sequencing (GBS) markers, gathered in the breeding population of an Austrian wheat breeding company, were available for the study. The dataset consisted of 3200 lines, genotyped for 7000 markers and tested in 68 trials. Linear mixed models and spatial adjustment was used to calculate trial heritabilities and adjusted genotypic means from the trial data. After filtering for trial heritability, the adjusted means were analysed in a second stage mixed model to calculate genotypic adjusted means per year, which were further used for the genomic analysis. A genome-wide association analysis was performed for each year to detect quantitative trait loci (QTL) associated with stripe rust. Genomic best linear unbiased predictor (GBLUP) models were employed to predict stripe rust within and across years. Genomic prediction within years was used to estimate the effects of training population size and marker density on the prediction performance. A model using previously found QTL as additional fixed effects (GBLUPA) was compared to the simpler GBLUP model. Genomic prediction across years was performed using all combi-

nations of available years as training and validation sets to evaluate the performance of genomic prediction in improving quantitative disease resistance.

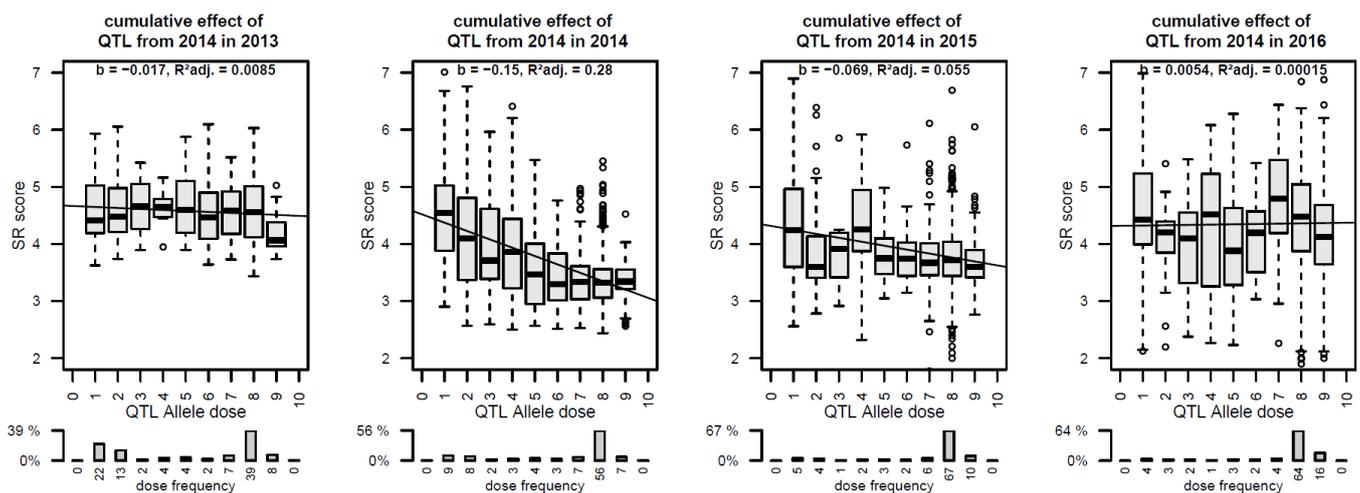
In the genome-wide association analysis, two large effect QTL were found. One QTL was found on chromosome 2A, largely fixed in the population and one was located on chromosome 2B, present in low frequency and therefore highly interesting for future breeding efforts. Both QTL were effective in 2014 and 2015, but not in 2013 and 2016 (Figure 1), indicating a shift in the prevalent stripe rust race. Genomic prediction performance was medium to high within years (Table 1), training population size and marker density were found to be sufficient for optimum prediction performance. As described in the literature, the GBLUPA model consistently outperformed the simpler GBLUP model, but only marginally. Prediction ability was low in most combinations of across years genomic prediction simulations, only combinations with 2014 and 2015 in the training or validation populations performed reasonably well. Variability in stripe rust scoring across years, but also within years and across trials, impaired the genomic prediction ability. This was further evidence for different races of stripe rust being present within and across years. Therefore, no clear conclusion was found towards the predictability of quantitative, non-race-specific resistance to stripe rust. Further research needs to focus on the development of concepts and models that can account for the adaptability of the pathogen to produce reliable predictions. Finding such a model can be challenging however, since it can only be validated in a population that does not carry major resistance genes. Additionally, a prediction model can only perform as well as the data that was used to train the model, when a model is trained with conflicting data the predictions will also be unreliable. This study also concluded, that breeders' data may bear strong variation ultimately impairing the power of genomic prediction models. Data has to be investigated thoroughly, and only the most informative trials should be chosen to train prediction models.

### Keywords

GBLUP · GWAS · *Puccinia striiformis* · QTL · *Triticum aestivum* · wheat breeding

**Table 1** Within year and across two years mean prediction abilities (PA) and standard deviations (SD). Calculated using full marker sets and randomly drawn training population sets.

Prediction	Training year	Validation year	Mean PA $\pm$ SD
Within year	2013	2013	0.53 $\pm$ 0.11
	2014	2014	0.70 $\pm$ 0.04
	2015	2015	0.38 $\pm$ 0.05
	2016	2016	0.58 $\pm$ 0.03
Across years	2013	2014	0.22 $\pm$ 0.07
	2013	2015	0.13 $\pm$ 0.02
	2013	2016	0.25 $\pm$ 0.03
	2014	2013	0.24 $\pm$ 0.02
	2014	2015	0.32 $\pm$ 0.01
	2014	2016	0.20 $\pm$ 0.01
	2015	2013	0.12 $\pm$ 0.03
	2015	2014	0.53 $\pm$ 0.04
	2015	2016	0.14 $\pm$ 0.04
	2016	2013	0.13 $\pm$ 0.03
	2016	2014	0.19 $\pm$ 0.01
	2016	2015	0.12 $\pm$ 0.01



**Figure 1** Allele dose effect of QTL found in 2014, compared across years. Box-plots show the distribution of phenotypes for each QTL allele bin combination, regression lines show the marker effect ( $b$ ) and  $R^2_{adj}$  denotes the total amount of phenotypic variance explained by QTL. Bar-plots show the distribution of allele doses in the respective population.

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## Selection of durum wheat lines under organic management - Preliminary results

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Organic cultivation has been increased over the last decades in whole Europe. Italy is among the five European countries with the largest acreage of organic farmland as well as among the five countries with more than 15% of agricultural land under organic management. Organic cultivation has several advantages such as decrease of soil erosion, decreased ecological impacts (*i.e.* biodiversity, nature conservation, water use efficiency, and environment), increased adaptiveness to climatic changes, but it has also some disadvantages which the most important is the reduced productivity. Genotypes under organic agriculture need a high phenotypic plasticity to adapt to different and changing climatic conditions, should be more tolerant to abiotic and biotic stresses, need a proper root system to enable efficient nutrient taking and therefore produce high enough yields with less/low input. Another important topic is salinity tolerance. More than 6% of the world surface is exposed to high salinity and drought risks, which are correlated and responsible for the decrease in agricultural land and crop production. Plants under salt stress suffer from two components of salt damage, *i.e.* osmotic stress and ion toxicity.

The H2020 project ECOBREED aims to select germplasm adapted to organic production. To fulfill this objective 72 durum wheat (*Triticum durum*) accessions were evaluated under organic management at the Tuscia University in Viterbo (latitude 42°25'7"68 N, longitude 12°6'34"20 E, altitude 300 m a.s.l.) in the 2018-2019 growing season. The accessions were chosen on the base of previous information and their potential for organic production. The germplasm included old varieties, local ecotypes, pre-breeding material and modern varieties. The accessions were characterized morpho-phenologically recording several characteristics such as the number of days to terminal spikelet, booting, stem elongation, heading, anthesis and grain filling. Moreover the following measurements were made: flag leaf width, flag leaf length, flag leaf area, plant height, spike dry weight at anthesis, biomass per shoot at anthesis, fertile floret number per spike at anthesis, spike length, spike index, number of spikelets per spike, number of grains per spike, number of grains per spikelet, grain yield per spike, spike fertility index, grain yield per square meter, number of grains per square meter, number of spikes per square meter, biomass per square meter, biomass per tiller, grain yield per tiller, 1000 grain weight, harvest index, chlorophyll content, grain protein, and gluten content. Some characteristics regarding the root system architecture were also recorded (*i.e.* root angle, depth, amount).

On the basis of the first year preliminary evaluation, 25 accessions out of the 72 screened accessions, were identified with positive yield and quality characteristics, tolerance to abiotic and biotic stresses, and variation in terms of phenological periods. Regarding phenology it should be noted that the accessions have shown different levels of precocity, but the ranking in terms of precocity is not the same across the different growth stages. Some accessions showed very fast juvenile growth while others have a faster development at later growth stages. This is of particular importance since nowadays, as a result of climate change, the environment is extremely erratic and it is important to grow germplasm with different precocity levels allowing to escape unpredictably periods of adverse conditions.

Experimental trials and molecular analyses were performed on the 25 accessions in order to identify genotypes tolerant to salt and drought stresses. We used molecular markers associated to genes and transcription factors (like TPS, DREB, WRKY, SOD, HKT) connected with metabolic pathways for salt tolerance, *i.e.* regulation mechanisms of osmoprotection and ion homeostasis. For drought tolerance molecular analyses were performed with markers (as GWM and WMS) associated with traits related to the root system. Data confirmed that marker WMS5 is linked to seminal root growth angle. Plants with deeper root systems are in general also earlier maturing while the ones with a more superficial root system seem to be later maturing. It should be also noted that the root system was different between the accessions of Central European origin and the ones from the Mediterranean area. Further evaluations in different locations and years are running to confirm the preliminary results.

### Keywords

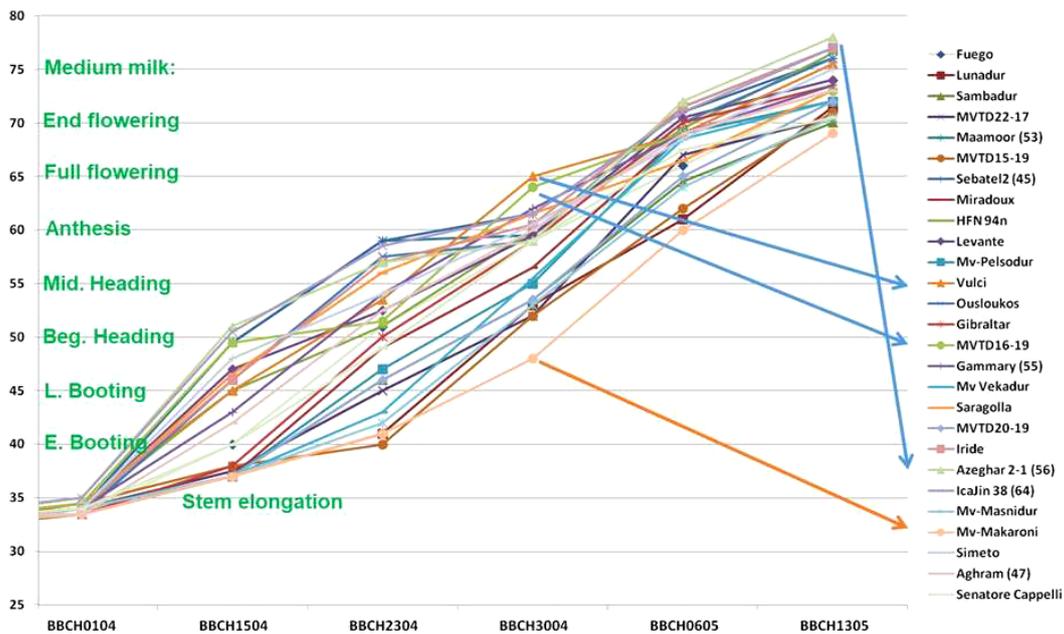
Drought stress · molecular marker · organic agriculture · root system architecture · salinity stress · *Triticum durum*

### Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 771367.



**Figure 1** Durum wheat accessions with contrasting root angles



**Figure 2** Differences in phenological development of durum wheat accessions. BBCH growth stage was scored on 1<sup>st</sup>, 15<sup>th</sup>, 23<sup>rd</sup> and 30<sup>th</sup> April, and on 6<sup>th</sup> and 13<sup>th</sup> May (x-axis) in Viterbo, Italy

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## WHEATSCAN – Did breeding from 1890 until today change the immunoreactivity of wheat?

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Wheat is one of the most important sources of nutrients for mankind and used in a huge variety of products for the food, feed and other sectors. However, wheat proteins are known to trigger wheat-related disorders including wheat allergy, celiac disease and non-celiac gluten sensitivity (NCGS) in predisposed individuals. Epidemiological evidence from several countries suggests an increasing prevalence of celiac disease and NCGS over the past decades, but the underlying reasons remain to be clarified. Next to increased awareness and better diagnostics, it may be possible that the human immune system has become more susceptible to developing allergic or autoimmunogenic disorders. Another factor that may have contributed are changes in the content and composition of immunoreactive proteins in wheat, e.g., due to fertilization or processing from flour to baked products. Wheat breeding with its goals to increase yield and resistance to abiotic and biotic stress factors might have inadvertently changed the immunoreactive profile of wheat, but there are only few studies on limited sets of different wheat varieties available. Therefore, our aim was to investigate changes in agronomic characteristics, protein content and protein composition of German winter wheat cultivars first registered from 1891 to 2010.

The five hexaploid wheat (*Triticum aestivum* L.) varieties most widely grown in Germany in each decade from 1891 to 2010 were sampled from the German Federal *ex situ* Genebank of crops at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK, Gatersleben, Germany). All varieties were grown together using an incomplete block design with three biological replications at Gatersleben in the harvest years 2015, 2016 and 2017. The grains from the three biological replications were pooled and milled into wholemeal flours. The protein content and composition were determined by the Dumas combustion method and modified Osborne fractionation combined with RP-HPLC-UV. The content of selected immunoreactive peptides was quantitated by LC-MS/MS. The results are presented as average of the three harvest years in the following.

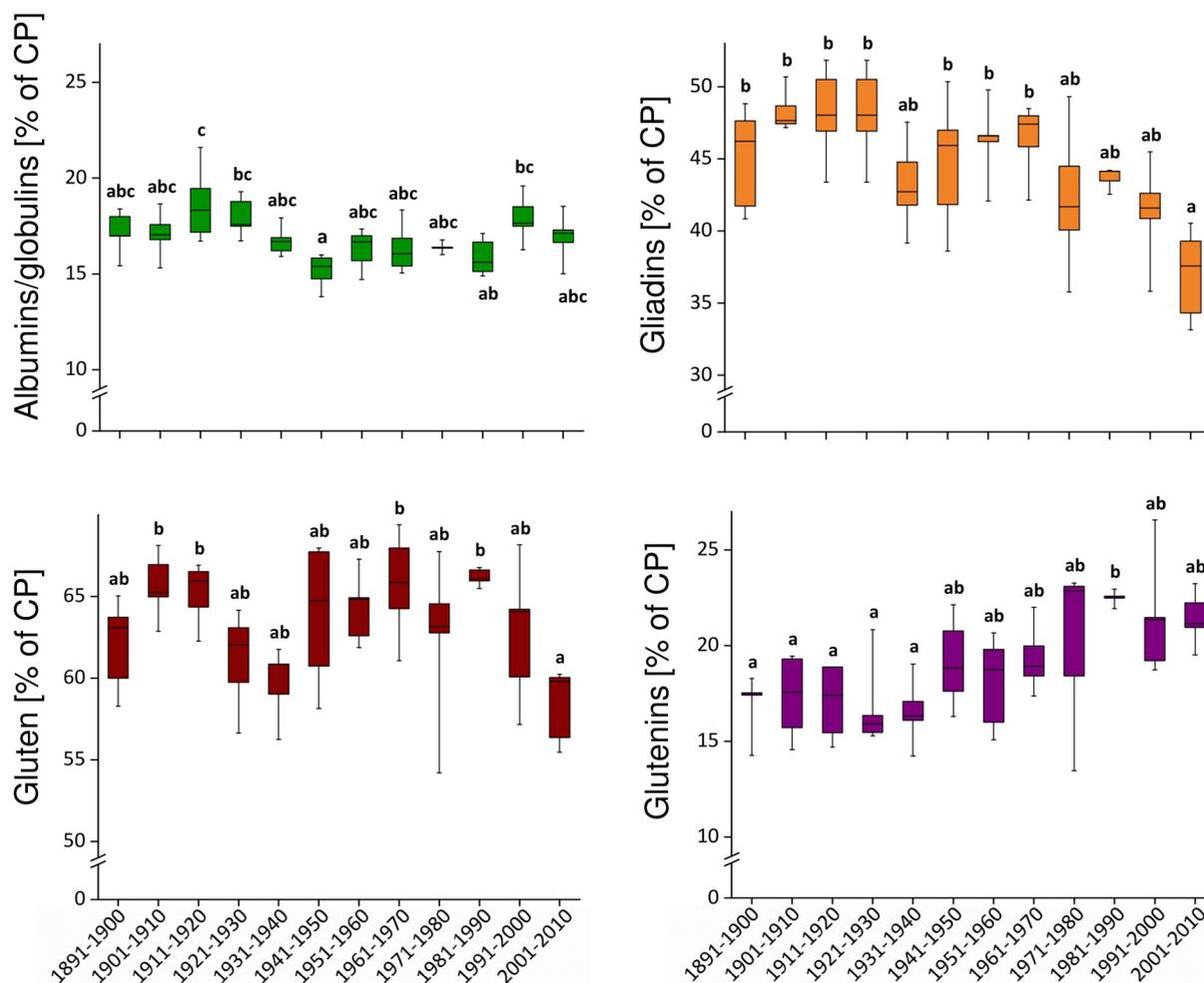
As expected, the plant height decreased from 117-153 cm (old varieties) to 76-99 cm (modern varieties). In contrast, the grain yield increased significantly over time from 31-49 dt ha<sup>-1</sup> to about 76 dt ha<sup>-1</sup>, as did the harvest index from about 0.40 to 0.58. The protein content showed high variation within the five varieties per decade and a slight decreasing trend from old to modern varieties, but the differences were not significant. The overall protein content was less than 10% in most samples, because the varieties were not fertilized. Regarding protein composition, the proportion of albumins/globulins was between 17% and 26%, but there was no change over time (Figure 1). The albumin/globulin fraction comprises water- and salt-soluble proteins and contains enzymes, enzyme inhibitors and other protective proteins, a number of which are also recognized as allergens. In the absence of any decreasing or increasing trend, the allergenic potential of the set of wheat varieties studied remained unaffected by breeding.

The proportions of gliadins decreased significantly over time from 62% to 46%, whereas those of glutenins increased significantly from 17% to 33%. In line with this, the gliadin/glutenin ratio decreased significantly from 3.6 to 1.4. The total gluten proportion and content did not show any significant changes over time, because gliadins decreased and glutenins increased, so that the sum of both remained largely unchanged (Figure 1).

The absolute content of four known immunoreactive peptides also showed no tendency to decrease or increase from old to modern varieties. Furthermore, the variability caused by environmental effects was higher than that caused by the genetic background of each variety. Taken together, all results point to the conclusion that the immunoreactive potential of old and modern wheat cultivars appears to be similar.

### Keywords

Celiac disease · gliadin · glutenin · non-celiac gluten sensitivity (NCGS) · *Triticum aestivum* · wheat allergy



**Figure 1** Content of albumins/globulins, gliadins, glutenins and gluten relative to the crude protein content (CP). Data are presented as median (line in the box) of 5 cultivars per decade (3 biological and 3 technical replicates), averaged over 3 harvest years 2015, 2016 and 2017, boxes represent the interquartile range, whiskers designate minima and maxima, different letters indicate significant differences between the decades (one-way ANOVA, Tukey's test at  $p < 0.05$ ).

## Acknowledgments

This research was funded by the Leibniz Association within the Leibniz Competition 2015, project number K195/2015 (WHEATSCAN: Unraveling the causes for wheat sensitivities).

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## Are 'ancient wheats' and old landraces better digestible?

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### Abstract

Wheat and especially modern cultivars are often associated with food intolerances and hypersensitivities. Gluten proteins, including gliadins and glutenins, as well as non-gluten proteins are known to provoke symptoms in susceptible individuals. Contrary to public assumption, recent studies demonstrated that modern wheat cultivars are even characterized by a lower immunogenic potential compared to old genotypes from the early 20<sup>th</sup> century due to reduced amounts of immunoreactive proteins. However, proteins of ancient wheat species (*e.g.* einkorn) and old common wheat landraces were found to release lower amounts of eliciting proteins after gastrointestinal digestion. On that account, einkorn in particular appears to be an alternative for people suffering from hypersensitivities, since also amylase-trypsin inhibitors, potential triggers of wheat sensitivity, are found to be largely absent.

### Keywords

Celiac disease · einkorn · gliadin · glutenin · hulled wheat · non-celiac gluten sensitivity (NCGS) · spelt · *Triticum aestivum* · wheat allergy

### Introduction

Nowadays, cereals and in particular wheat have a bad medial reputation, resulting in a lower consumption of these important staple foods. Nutritional trends such as 'gluten free' further promoted this progress. More than 20 different wheat (sub)species are described, which differ in their number of genomes and can be classified into three groups: the diploid einkorn group with only one genome (AA,  $2n=2x=14$ ), the tetraploid emmer group with two genomes (BBAA,  $2n=4x=28$ ) and the hexaploid spelt group with three genomes (BBAADD,  $2n=6x=42$ ) (Schulz 1913, Sakamura 1918). Einkorn (*Triticum monococcum*), emmer (*T. dicoccum*) and spelt (*T. spelta*) are hulled cultivated forms of these groups, that means the grains are covered by the husk and a further processing

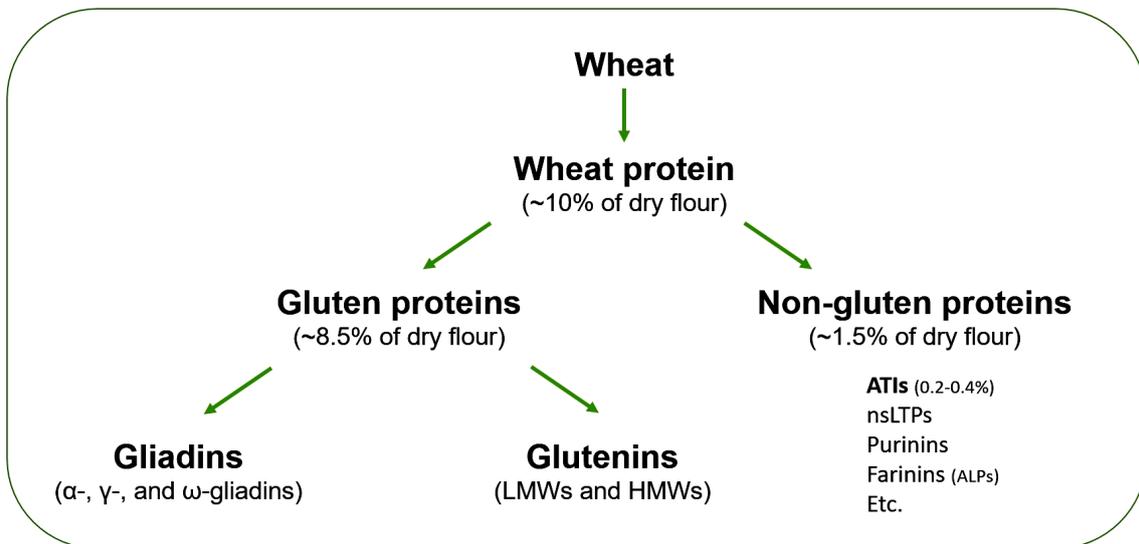
step, *i.e.* dehulling, is needed to remove the glumes before milling. The tetraploid durum (*T. durum*) and hexaploid bread or common wheat (*T. aestivum*) represent free-threshing species of the mentioned groups (Figure 1). Besides common wheat, responsible for approximately 90% of the global wheat acreage, only durum has an economic relevance. All other wheat species are of only regional importance. Yields of common wheat and durum were significantly increased by breeding during the last century. The other wheat species have distinctly lower yields, but are better adapted to organic crop management due to a better weed competitiveness resulting from increased plant height, and less requirements of nutrients and soil fertility (Janovská et al. 2021).

Wheat is often associated with food intolerances and disorders (Table 1) which might contribute to its bad image. The pathogenesis, their triggers and mechanisms of coeliac disease (CD) and wheat allergy are well understood, even specific biomarkers for their diagnosis are known. Contrary, the diagnosis of wheat sensitivity is far more complicated and difficult. On the one hand, symptoms like bloating, digestive problems and headache are similar to the previously mentioned disorders, on the other hand, specific biomarkers for diagnosis are missing. The clinical diagnosis of wheat sensitivity is predominately based on the exclusion of other disorders combined with an alternating diet, with and without gluten containing cereals (Kissing Kucek et al. 2015, Scherf 2019).

Novel studies revealed that so called amylase-trypsin inhibitors (ATIs) promote gastrointestinal inflammation and thus are mainly responsible for wheat sensitivity. Probably, gluten and fructans play a distinct role as well. Fructans are oligosaccharides mainly built up of fructose and belong to the group of FODMAPs (Fermentable Oligo-, Di-, Monosaccharides And Polyols). It is assumed that FODMAPs at least contribute to symptoms. IBS (irritable bowel syndrome) is a further gastrointestinal indisposition and the triggers are manifold with FODMAPs and allergenic proteins being potential triggers as well. The majority of IBS pa-



**Figure 1** Wheat species cultivated in Austria: **a** einkorn (*Triticum monococcum*), **b** emmer (*T. dicoccum*), **c** durum (*T. durum*), **d** spelt (*T. spelta*), and **e** common wheat (*T. aestivum*). Einkorn, emmer and spelt are hulled, bread wheat and durum are free-threshing wheats. (Source: modified based on single pictures from the Virtual Cereal Cultivar Garden, Institute for Plant Sciences of the ETH Zurich; <http://www.sortengarten.ethz.ch>)



**Figure 2** Composition of wheat storage protein (ALPs, avenin-like proteins; ATIs, amylase-trypsin-inhibitors; HMW, high molecular weight; LMW, low molecular weight; nsLTGs, non-specific lipid-transfer proteins)

tients respond well to a low-FODMAP diet. Many symptoms of different disorders caused by the consumption of wheat are very similar. Furthermore, the same proteins and carbohydrates are involved in their pathomechanisms, which makes a diagnosis even more difficult. Totally, up to 10% of the population cannot tolerate wheat products due to mentioned ingredients (see Table 1) (Kissing Kucek *et al.* 2015, Scherf 2019, Janovská *et al.* 2021).

### Results and discussion

The proteins in wheat are very complex and can be divided into two groups, the non-gluten and the gluten proteins, namely gliadins (α-, γ-, and ω-gliadins) and glutenins (LMWs and HMWs) (Figure 2). The knowledge about gluten is enormous, mainly be-

cause of its excellent technological properties, whereas little is known about the non-gluten proteins. ATIs, nsLTGs (non-specific lipid-transfer proteins), purinins, farinins (ALPs, avenin-like proteins), etc. belong to this group and together with other prolamines represent an important class of allergens called 'prolamine superfamily' (Radauer & Breiteneder 2007).

In respect to wheat sensitivity research was focused on ATIs, which protect the plants due to their inhibition towards amylases and proteases. Today, 19 different protein isoforms of ATIs are known, which can be classified into monomeric, dimeric and tetrameric forms (Scherf 2019). A prejudice is that breeding resulted in elevated ATI contents in modern cultivars and thus is responsible for an increased number of people suffering from wheat sensitivity.

**Table 1** Overview of wheat related disorders (Source: Kissing Kucek *et al.* 2015, Scherf 2019)

	Coeliac disease	Wheat allergy <sup>1</sup>	Wheat sensitivity
Prevalence	0.5 – 2%	0.2 – 0.5%	0.6 - 6%
Pathogenesis	autoimmune	IgE induced	unspecific immune based
Marker	IgA anti-EMA, IgA anti-tTG, IgG anti-DGP, IgA anti-gliadin	specific IgE antibodies	IgA/IgG anti-gliadin in 50% of cases
Genetic Preposition	>95% DQ2-DQ8 HLA positive	DQ2-DQ8 HLA negative	≈50% DQ2-DQ8 HLA positive
Uptake	oral	oral, respiratory & percutaneous	oral
Allergen/Antigen	peptides from gliadins and glutelins	depending on type of allergy gliadins, glutelins and/or non-gluten proteins (e.g. ATIs)	ATIs (possibly gluten and FOD-MAPs, predominately fructans)

<sup>1</sup> WDEIA (wheat dependent exercise induced anaphylaxis), contact-urticaria and baker's asthma are summarized under 'wheat allergy'

ty. Call *et al.* (2020) examined Austrian wheat cultivars from different periods, from the 19<sup>th</sup> century to the 2010s, and showed that there was no increased ATI accumulation in modern wheat cultivars. However, a high variability of ATI concentrations was observed within the grains of the same time period. Thus, breeding of low ATI cultivars seems to be possible. The same study also analyzed other wheat species besides common wheat, including einkorn, emmer, durum and spelt. A relationship between ploidy level and ATI amounts was detected. Hexaploid spelt and bread wheat revealed significantly higher ATI contents, whereas einkorn only showed very low amounts of ATIs and tetraploid emmer and durum showed moderate ATI levels. The exceptional position of einkorn was confirmed by Geisslitz *et al.* (2020) who showed marginal ATI concentrations and the complete missing of some specific ATIs. Furthermore, the mentioned correlation between ploidy level and occurrence of ATIs was found as well, but with less coherence. In respect to other non-gluten proteins little research activities were performed so far.

Additionally, Call *et al.* (2020) studied ATI and gluten compositions. The results revealed that modern cultivars are enriched in HMWs, which are essential for good baking performance. Contrary, contents of gliadins, especially  $\gamma$ -gliadins, were reduced in the last century. Thus, the gliadins to glutenins ratio is inferior in respect to baking quality for old landraces from the 19<sup>th</sup> century. Summing up, modern bread wheats have superior baking properties and a slightly reduced immunogenic potential. A similar trend in respect to protein composition was observed for other wheat species. Durum and common wheat showed significantly higher glutenin and HMW amounts than spelt, emmer and einkorn. Due to these results the wheat species can be ranked according to the baking quality: bread wheat > spelt > durum > emmer and einkorn. The inferior ratio of gliadins and glutenins of hulled wheats can be partially compensated by higher protein contents. However, the described shift in gluten composition provokes an increased immunogenic potential of ancient wheats (Geisslitz *et al.* 2018, Call *et al.* 2020, Janovská *et al.* 2021).

Besides the varying protein composition, gluten proteins from different wheat species showed contrasting behaviors when digested. Einkorn and durum showed a strongly reduced number of T-cell epitopes (which can promote CD) after digestion compared to bread wheat (Janovská *et al.* 2021). Asledottir *et al.* (2020) ana-

lyzed peptides after gastrointestinal *ex vivo* digestion and found that hulled wheats released a distinctly lower number of peptides containing epitopes relevant in CD. Similar results but with less divergence in number of harmful epitopes were obtained for landraces when compared to modern *T. aestivum* cultivars. Although all common wheat accessions contained polypeptides with strong immunogenic potential (*e.g.* 33mer) in high amounts, old landraces released fewer peptides triggering CD after digestion. This study clearly pointed out that not the quantity of immunogenic proteins alone, but their degradation by digestive enzymes is crucial for the induction of adverse reactions.

### Conclusion

Old common wheat cultivars and landraces did not show any indication to be better tolerable based on their protein composition. The amount of ATIs is comparable to modern cultivars. The share of gliadins is even higher, whereas lower amounts of glutenins were measured, which are most important for the baking quality. These results suggested that old landraces even have an increased immunogenic potential. Nevertheless, hulled wheat species, especially einkorn, seemed to be less harmful for people suffering from wheat sensitivity, mainly due to their distinctly lower ATI occurrence. Spelt seemed to be no alternative for people with wheat sensitivity, because of the high abundance of ATIs. Nevertheless, recent studies showed strong differences in hydrolysis of proteins by proteases among different wheat species and common wheat cultivars from different breeding periods. These novel findings even suggest that not the concentration of proteins, but their degradation by digestive enzymes is crucial for their immunogenic potential. Any explanation by structural differences of the mentioned proteins is totally missing so far. Thus, further and more intensive studies have to be conducted in the future to verify the predominant role of digestion in respect to the immunogenic potential of wheat proteins. Anyhow, no wheat cultivar or species is suitable for people with coeliac disease or wheat allergy, because proteins with high allergenic potential are found in any wheat.

### Acknowledgments

This research was funded by FFG (Austrian Research Promotion Agency), project ID-WHEAT (FFG project no. 858540).

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## Influence of water addition on baking properties of organic wheat

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Results of Austrian conventional and organic wheat VCU (value for cultivation and use) trials challenged the suitability of tests hitherto used for the evaluation of the baking quality of common wheat (*Triticum aestivum*) for both management systems. Of special interest in this context is the optimal water absorption/addition with respect to baking volume. Five wheat varieties (i.e. 'Arnold', 'Aurelius', 'Bernstein', 'Capo', 'Emotion') were grown on 12 Austrian test sites which were located in the main wheat producing areas of the Pre-Alpine foothills and the Pannonian plains, six each under conventional and organic management. Equal amounts of grains from each site were used for milling by a Bühler MLU 202 laboratory mill in order to create a conventional as well as an organic flour mix of each variety. Subsequently the flour samples were chemically analysed for ash content, grain hardness, crude protein and wet gluten content, Zeleny sedimentation value, content of damaged starch and the individual Osborne protein fractions. Rheological tests were carried out using the farinograph and the extensograph. Loaf volume was determined by the traditional 'Kaisersemel' baking test using +1% and +3% water addition on the basis of the extensograph water absorption. Chemical and rheological properties showed a more pronounced effect of the management system (Table 1), whereas the differences between the varieties were smaller and partly subject to significant genotype by management interaction. Considering protein fractions, conventional production significantly increased the gliadin content, while for glutenins no or only a slight increase was observed for conventional grown samples with the exception of 'Arnold'. This also led also to a continuous higher gliadin to glutenin ratio of conventional samples. No influence of the management system was observed on flour yield, grain hardness, ash content and content of damaged starch. Also, damaged starch content was not correlated with grain hardness. Wet gluten content highly correlated with crude protein content ( $r=0.92-0.94$ ,  $p<0.01$ ), which was mainly due to the high correlation to gliadins ( $r=0.77-0.87$ ,  $p<0.01$ ) while the correlation with glutenins was not significant. Contrary, Zeleny sedimentation volume highly correlated with glutenins ( $r=0.73-0.77$ ,  $p<0.01$ ). While under conventional management a significant correlation was observed both with low (LMW) and high molecular weight (HMW) glutenins ( $r=0.75$ ,  $p<0.01$ ), organic samples showed a significant correlation only between LMW and sedimentation volume ( $r=0.84$ ,  $p<0.01$ ).

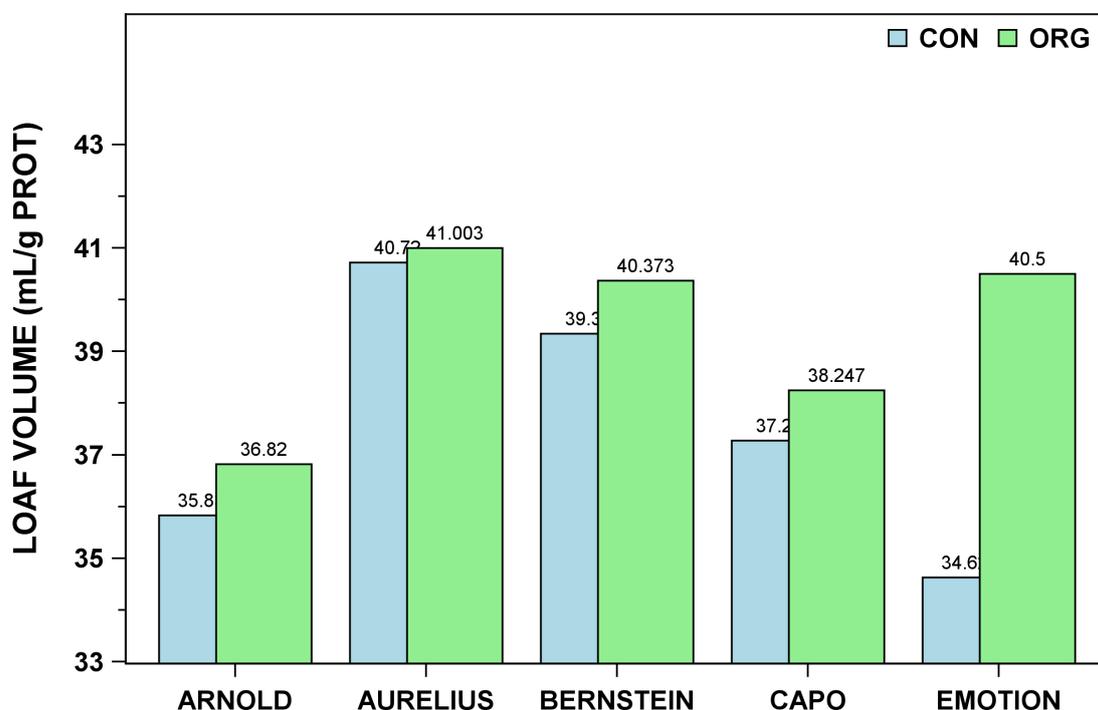
Farinograph stability and quality number were generally higher in conventional samples with the exception of 'Emotion' which showed the opposite reaction. For extensograph parameters the influence of the management system was dissimilar: extensibility was higher in conventional samples, while resistance was higher in organic samples. This reflects well the abundance of gliadins and glutenins in the two management systems, their ratio and their properties for the different extensograph parameters with gliadins being related to dough extensibility while glutenins being related to dough strength. Water absorption was subject to genotype by management interaction with 'Arnold', 'Bernstein' and 'Emotion' showing significant higher absorption for conventional samples, while 'Aurelius' and 'Capo' showed a slightly higher absorption in the organic samples. Loaf volumes were generally higher for conventional samples, however, for 'Emotion' this effect was less pronounced. A higher water addition of +3% did not result in higher loaf volumes for almost all genotypes. Only 'Bernstein' benefited from a higher water addition and realized higher loaf volumes for both the conventional and organic sample. Expressing the loaf volumes as volumes realized per gram crude protein it became evident that organic protein is more efficiently relocated into loaf volume. This effect was especially pronounced for 'Emotion' (conventional: 34.6 mL/g protein; organic: 40.5 mL/g) (Figure 1). From the baking tests it can be concluded that the current classification scheme according to the Austrian Descriptive List of Varieties exactly describes the baking quality of conventional samples. However, the loaf volumes realized for the organic samples were not in accordance with the current baking quality classification (Figure 2). The currently used methods are, therefore, not adequately addressing the quality of organically grown wheat.

### Keywords

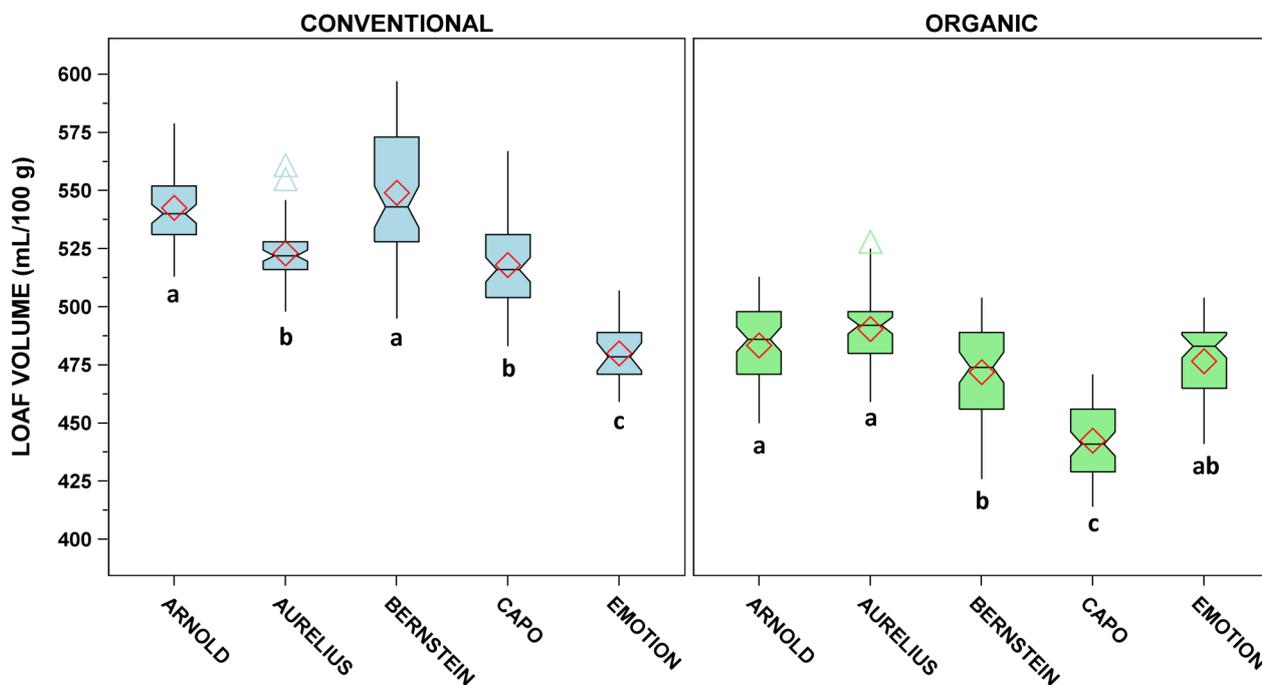
Baking quality · loaf volume · organic breeding · protein · *Triticum aestivum* · VCU test · water absorption

**Table 1** Genotype by management means for selected quality traits (Means with different letters within a management system are significantly different at  $p < 0.05$ ; BQ = baking quality classification according to the Austrian Descriptive List of Varieties; con = conventional management; org = organic management; Ext. = extensograph; Far. = farinograph).

Variety	BQ	Crude protein (%)		Gliadins (g/100 g)		Glutenins (g/100 g)		Gliadin:glutenin ratio	
		con	org	con	org	con	org	con	org
Arnold	8	15.2 a	13.1 a	7.47 a	5.98 a	6.30 a	4.26 a	1.19 b	1.40 a
Aurelius	7	12.8 c	12.0 b	5.16 c	4.12 b	4.83 b	4.67 a	1.07 b	0.88 c
Bernstein	8	14.0 b	11.7 cd	5.80 b	3.55 c	5.15 b	3.90 a	1.13 b	0.91 c
Capo	7	14.0 b	11.6 d	7.23 a	4.45 b	5.25 b	4.29 a	1.38 b	0.91 bc
Emotion	6	14.0 b	11.8 c	5.73 bc	4.39 b	3.40 c	3.37 a	1.69 a	1.38 ab
		Sedimentation value (mL)		Far. stability (min)		Far. quality number		Far. water absorption (%)	
		con	org	con	org	con	org	con	org
Arnold	8	54.3 b	43.7 a	14.5 a	8.4 a	160 a	100 a	64.9 a	62.9 a
Aurelius	7	51.7 c	45.3 a	9.7 b	8.2 a	98 bc	81 b	57.7 d	58.3 d
Bernstein	8	58.0 a	44.0 a	10.3 b	5.9 b	116 b	63 c	60.9 c	58.5 d
Capo	7	49.7 c	36.7 b	7.3 c	5.8 b	98 bc	67 c	61.2 c	61.7 b
Emotion	6	36.7 d	29.3 c	5.1 d	6.7 b	82 c	87 b	62.9 b	60.4 c
		Ext. extensibility (mm)		Ext. resistance 5 min (EU)		Loaf vol. (mL/100 g flour)		Loaf volume (mL/g protein)	
		con	org	con	org	con	org	con	org
Arnold	8	172 a	167 a	370 b	387 b	544 a	483 a	35.8 d	36.8 c
Aurelius	7	151 b	143 b	486 a	517 a	522 b	491 a	40.7 a	41.0 a
Bernstein	8	163 a	124 bc	505 a	571 a	552 a	472 b	39.3 b	40.4 a
Capo	7	157 b	138 b	386 b	406 b	521 b	442 c	37.2 c	38.2 b
Emotion	6	132 c	116 c	261 c	353 b	484 c	477 ab	34.6 e	40.5 a



**Figure 1** Loaf volume realized per gram crude protein: organic protein is more efficiently relocated into loaf volume with an especially pronounced effect for 'Emotion'. For statistical mean comparisons see Table 1.



**Figure 2** Genotypic variability in loaf volume subject to the management system (Means are indicated by red diamonds and are significantly different at  $p < 0.05$  within the respective management system if labelled by different letters).

#### Acknowledgments

This research was funded by a scholarship of ICC Austria to S.L.

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## Genetic structure of a germplasm for hybrid breeding in rye (*Secale cereale* L.)

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### Abstract

Rye (*Secale cereale* L.) responds strongly to changes in heterozygosity with hybrids portraying strong heterosis on all developmental and yielding characteristics. In order to achieve the highest potential heterosis, parental lines must originate from genetically distinct gene pools. Here we report the first comprehensive SNP-based study of genetic diversity and population structure in a 'Gülzow' (G) cytoplasm based hybrid rye breeding germplasm. In total 365 inbred lines from Nordic Seed Germany were genotyped for 4419 polymorphic SNPs. The aim of this study was to confirm and quantify the genetic separation of parental populations, unveil their genetic characteristics and investigate underlying population structures. Through a palette of complementing analysis, we confirmed a strong genetic differentiation ( $F_{ST} = 0.332$ ) of parental populations validating the germplasms suitability for hybrid breeding. These were, furthermore, found to diverge considerably in several features with the maternal population portraying a strong population structure characterized by a narrow genetic profile, small effective population size and high genome-wide linkage disequilibrium. We propose that the employed male-sterility system putatively constitutes a population determining parameter by influencing the rate of introducing novel genetic variation to the parental populations. Findings of our study emphasized the immediate value of comprehensive population studies on elite breeding germplasms as a pre-requisite for application of genomic-based breeding techniques, introgression of novel material and to support breeder decisionmaking.

### Keywords

Effective population size · genetic diversity · hybrid breeding · phylogenetic analysis · population structure

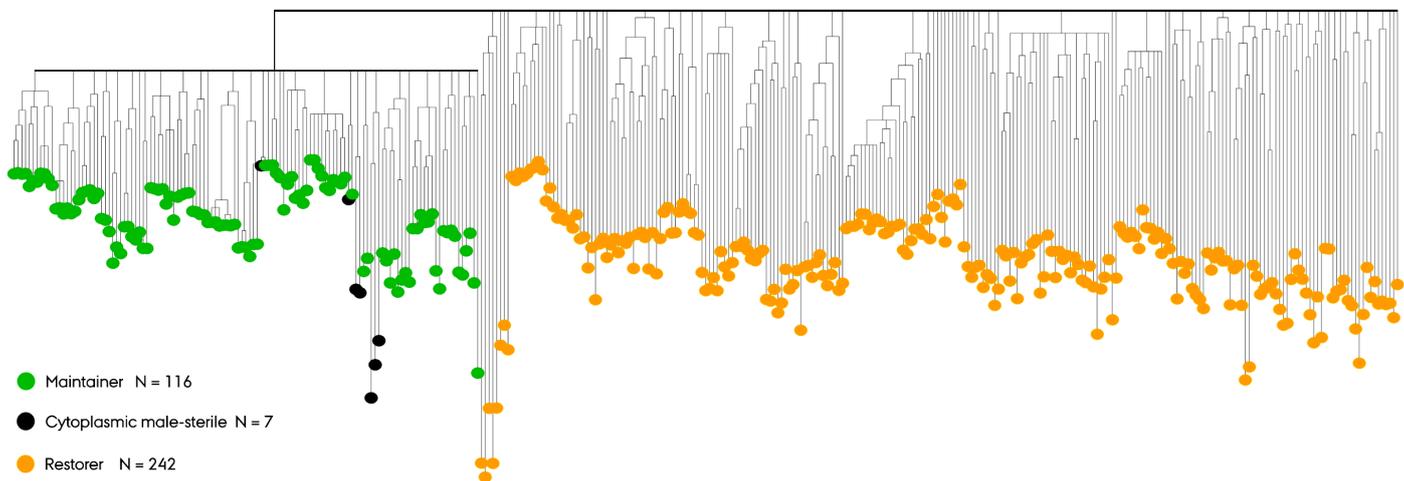
### Introduction

Rye (*Secale cereale* L.) remains to this day a prominent crop in the temperate northern hemisphere recognized for its tolerance to abiotic and biotic stresses, demonstrating superior yield potential under marginal conditions. As a cross-fertilizing crop species, self-compatibility has only recently been introduced in cultivated rye rendering inbred lines predisposed to severe inbreeding depression. Rye therefore responds strongly to changes in heterozygosity with hybrids demonstrating strong heterotic effects on all developmental and yield characteristics. In order to achieve the highest potential heterosis, parental lines must originate from genetically distinct gene pools. The majority of hybrids originate from the predominant 'Petkus' and 'Carsten' gene pools deploying the Pampa (P) type cytoplasmic male sterility (CMS) system. The P-type system is inherently characterized by the low frequency of gametes carrying effective restorer alleles resulting in inadequate and environmental unstable restoration of male-fertility. In contrary, the less predominant 'Gülzow' (G) type CMS system exhibits superior restoration of male fertility. Here we report the first comprehensive SNP-based study of genetic diversity and population structure in a G type based hybrid rye elite breeding germplasm. The aim of this study was to confirm and quantify the genetic separation of parental populations, unveil their genetic characteristics and investigate underlying population structures.

### Material and methods

#### Plant material

In total 365 inbred lines comprised of 242 restorers, 116 non-restorer germplasm (NRG) and 7 CMS lines from Nordic Seed Germany GmbH were selected for the study. The restorer lines originated from the 'Petkus' and 'Carsten' gene pools with a predominance of the latter suggested by available pedigree data, whereas information on the precise origin of the NRG lines is not-existent. The CMS male sterility is based on the 'Gülzow' type cytoplasm originating from the Austrian population rye variety 'Schläger alt' (Melz & Adolf 1991).



**Figure 1** Neighbor-joining dendrogram of the Nordic Seed hybrid rye elite breeding germplasm ( $N_{pop} = 365$ ), based on 4419 SNP markers

### Molecular marker and SNP genotyping

Samples were genotyped using a pre-designed Illumina Infinium 15Kwheat and 5KRye SNP iSelect ultra HD chip. Rye specific markers comprised 2698 markers from the 5K array by Haseneyer *et al.* (2011) and 2059 markers from the 600K array by Bauer *et al.* (2017). Markers were prior to analysis filtered for marker allele frequency  $\geq 0.005$ , missing individual score  $\leq 0.2$ , and, missing marker score of  $\leq 0.1$ .

### Data analysis

Population genetic analysis of SNP marker data was done in R studio (v. 1.1.463) interface in R statistical software (v. 3.6.3) by application of various predesigned packages. Analysis included calculation of genetic characteristics, principal component analysis, admixture model of inferred ancestry, phylogenetic analysis and linkage disequilibrium.

### Results and discussion

Through a palette of complimenting analysis, we confirmed a strong genetic differentiation ( $F_{ST} = 0.332$ ) of parental populations validating the G-type CMS based germplasms suitability for hybrid breeding (Figure 1). Using 4419 informative SNP markers the populations were found to diverge considerably in several key genetic characteristics. The maternal NRG and CMS population was characterized by a narrow genetic profile ( $H_s = 0.250$ ), small effective population size ( $N_e = 72$ ), and high interchromosomal linkage disequilibrium ( $LD_{mean} = 0.379$ ). The paternal R population was characterized by a broader genetic profile ( $H_s = 0.333$ ), larger effective population size ( $N_e = 137$ ), and lower interchromosomal linkage disequilibrium ( $LD_{mean} = 0.177$ ). A detailed account of the findings can be found described by Vendelbo *et al.* (2020). Contrary to the P-type CMS system, the G-type is characterized by a low frequency of gametes carrying the NRG cytoplasm. On basis of our findings we propose that the employed male-sterility system putatively constitutes a population determining parameter by influencing the rate of introducing novel genetic variation to the parental populations. Findings of our study emphasized the immediate value of comprehensive population studies on elite breeding germplasms as a pre-requisite for application of genomic-based breeding techniques, introgression of novel material and to support breeder decision-making.

### Acknowledgements

We would like to thank laboratory technician Hanne Svenstrup at Nordic Seed A/S for contribution to the genotypic data collection. The plant material included in this study was provided by Nordic Seed Germany GmbH (Nienstädt, Germany). The high throughput SNP genotyping was performed by Trait Genetics (Gatersleben, Germany).

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## Application of transcriptomics to decipher detoxification of fusariotoxins in the grain of rye (*Secale cereale* L.) hybrids

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RNA sequencing combined with innovative bioinformatic tools can identify candidate genes and can find the functional elements useful for practical breeding. The aim of this study was to characterize the transcriptome of rye after *Fusarium* head blight (FHB) infection. The rye hybrids included in this study accumulated contrasting levels of fusariotoxins after pathogen inoculation. Moreover, both hybrids consist of similar genetic components except the restorer component. Therefore, they provided a good experimental set up to decipher detoxification mechanism of fusariotoxins. Detoxification of fusariotoxin is a type V of FHB resistance and considered a component of Type II resistance which is related to spread of infection within spikes. Understanding this type of resistance is vital for FHB resistance, but to date nothing is known about candidate genes that confer this resistance in rye due to scarce genomic resources.

In this study, we established field trials involving two rye hybrids, *i.e.* 'Helltop' and 'DH372'. Both rye hybrids were tested for their response to *Fusarium* spp. under optimal infection conditions. Two *Fusarium* isolates, *i.e.* *F. graminearum* (strain 7775) and *F. culmorum*, common in Denmark and known producers of deoxynivalenol (DON) were used for inoculation. The disease severity of FHB was assessed and fusariotoxin accumulation in the seeds of inoculated hybrids were quantified in a LC-MS/MS system. An experiment to obtain plant material for a comparative transcriptome analysis was conducted in environment-controlled growth chambers. The mRNA of samples was fragmented and transformed to 100 bp short insert strand specific cDNA libraries for sequencing on DNBseq PE100 from BGI (Europe). The subsequent workflow was as follows: identification of differentially expressed genes (DEGs), gene mining, *in silico* analysis of DEGs, biological pathways, identification of conserved regulatory elements and associate them to specific transcription factors (TFs).

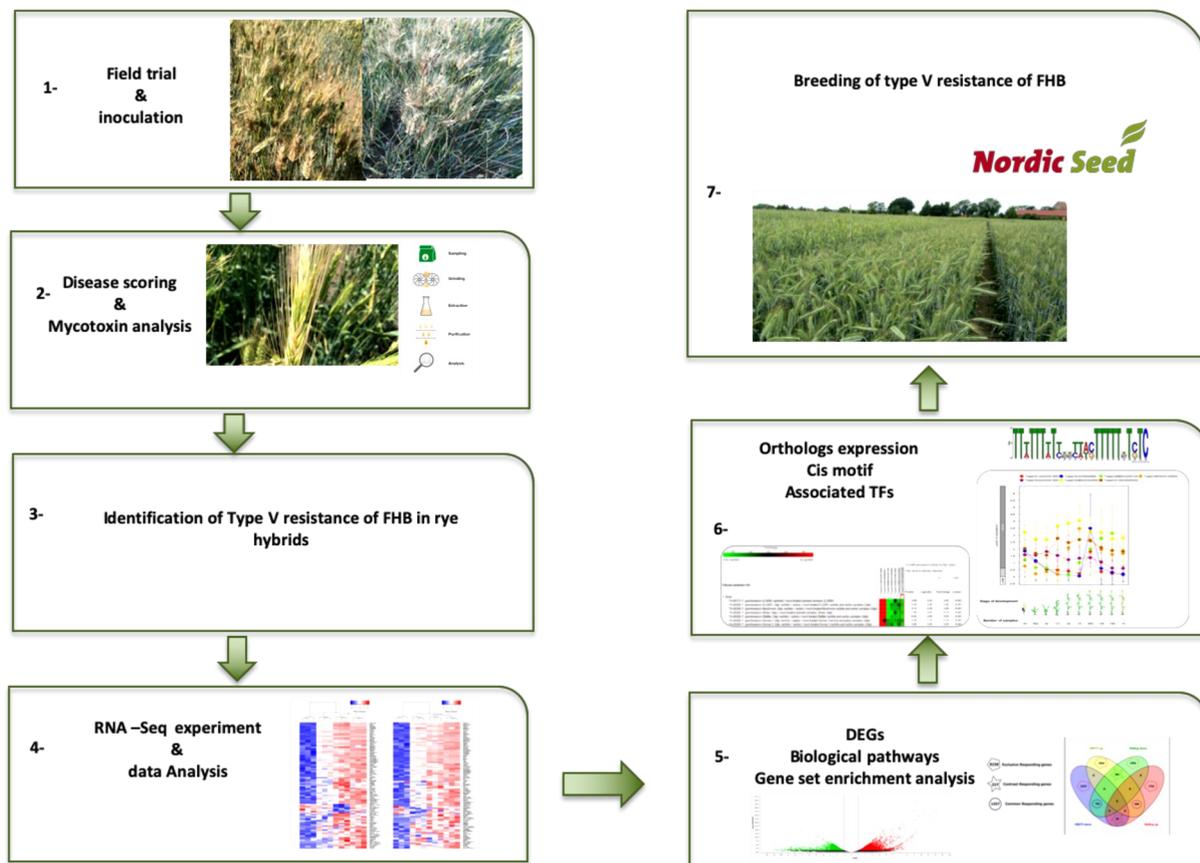
Gene mining identified candidate genes and pathways contributing to the detoxification of fusariotoxins in rye. In the fusariotoxin analysis, we found that grain from the Nordic Seed rye hybrid

'Helltop' accumulated 4 times higher concentrations of DON, 9 times higher nivalenol (NIV) and 28 times higher contents of zearalenone (ZEN) than the hybrid 'DH372' after artificial inoculation under field conditions. In the transcriptome analysis, we identified 6675 and 5151 DEGs in 'DH372' and 'Helltop', respectively, compared to non-inoculated control plants. A Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that DEGs associated with glycolysis and the mTOR signaling pathway in 'Helltop', whereas carbon fixation in photosynthesis organisms were represented in 'DH372'. The gene ontology (GO) enrichment and gene set enrichment analysis (GSEA) of DEGs lead to the identification of the metabolic and biosynthetic processes of peptides and amides in 'DH372', whereas photosynthesis, negative regulation of catalytic activity and protein-chromophore linkage were the significant pathways in 'Helltop'. In the process of gene mining, we found four genes that were known to be involved in FHB resistance in wheat and that were differentially expressed after infection only in 'DH372' but not in 'Helltop'. The transcriptomics data generated in this study will be a valuable asset to enhance overall omics resources for rye, particularly keeping in view that previously there is not a single study in rye aiming to decipher FHB resistance through utilizing transcriptomic profiling.

The results of this study will be useful for rye breeders to develop strategies against type V of FHB. Moreover, accessibility of such transcriptome sets under *Fusarium* stress as offered here will serve as precious resource for rye breeders and to the research community for further functional and comparative genomics studies in rye.

### Keywords

*Cis*-regulatory elements · disease resistance · GO enrichment · rye hybrids · transcriptome



**Figure 1** Workflow of experimental set up, data analysis and findings of the transcriptomics work to decipher detoxification of fusariotoxins in the grain of rye hybrids

### Acknowledgements

This work was funded by Innovation Fund Denmark (7039-00016B) and Pajbjerg Foundation

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## Association genetics of Fusarium head blight and deoxynivalenol resistance in *Aegilops tauschii*

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Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the most important diseases of wheat. It not only results in yield loss, but also reduces the quality due to contamination with mycotoxins, especially deoxynivalenol (DON). Exploring resistance genes from the wild relative *Aegilops tauschii* is a promising strategy to improve wheat resistance. This study aims to evaluate the variation for FHB and DON resistance within a diversity panel of *Ae. tauschii* accessions and to detect variants at genomic loci associated with FHB and DON resistance. Therefore 147 *Ae. tauschii* accessions were point-inoculated by pipetting *F. graminearum* spores into a single spikelet per head and the spreading of the disease was determined. In parallel, the heads were also infiltrated with DON and the mycotoxin-induced bleaching symptoms were recorded. We revealed broad variation of the FHB spreading within the spike, although no highly resistant accessions were identified and FHB spreading promoted early 'spike shattering' thereby complicating disease phenotyping. For DON resistance less variation was found, most of the accessions showed bleaching symptoms only on the treated spikelet, but for nine genotypes the DON infiltration resulted in severe bleaching symptoms. DON contents and the conjugated non-toxic DON-3-O-glucoside (D3G) contents of treated heads showed that the mechanism of DON resistance in *Ae. tauschii* is through glycosylation process by converting DON to D3G that has lower toxicity properties. Association genetics based on k-mers identified two highly significant peaks on chromosome 5D. The peaks were physically located in the reference 'Chinese Spring' (228.9-256 Mb) comprising 129 genes.

### Keywords

Association genetics · *Fusarium graminearum* · mycotoxin · wheat wild relative

### Acknowledgements

Funding by the Ministry of Education and Culture of the Republic of Indonesia and the Austrian Agency for International Cooperation in Education and Research (OeAD-GmbH) in cooperation with ASEAN-UNET is acknowledged.

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## ProFaba - Towards genomics-based breeding in faba bean

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Europe currently has a substantial deficit in plant protein production, especially when it comes to crops with high protein content. This deficit is covered by soy import, leading to an unbalanced agricultural system that relies on import of both plant protein and mineral fertilizers. With an average protein content of 29%, faba bean (*Vicia faba*) is a prime candidate for a widely adapted protein crop that could substantially contribute to reducing soy imports by increasing the local and sustainable production of plant protein in Europe.

ProFaba has brought together faba bean researchers engaged in a number of national and international projects, including NORFAB, Papugeno, BEANS4N.AFRICA, PeaMUST, EUCLEG and Abo-Vici. We carry out multi-site trials using two common germplasm collec-

tions. A broad, general panel, ProFaba200, and the Göttingen winter bean panel, which is dedicated to studying frost tolerance. Both panels have been genotyped using an Axiom array developed at the University of Reading. The trials provide us with quantitative data on yield, disease and agronomic traits that are collected in a common Faba Base repository (<https://faba.au.dk>), which we will use for analysis of genotype by environment interactions and phenological modelling, allowing identification of potential climate change impact on European faba bean cultivation. In addition, we conduct in-depth phenotyping and detailed genetic investigation of specific traits of interest, including autofertility, disease resistance, bruchid-, acid- and frost tolerance, rhizobium interactions and protein quality. We have found significant variation for all traits of interest within the ProFaba200 and Göttingen winter

bean diversity panels and genetic analyses are ongoing. ProFaba partners have also nucleated a faba bean genome sequencing consortium (<https://fabagenome.dk>), and marker and genotype information will be integrated with the faba reference genome sequence as it becomes available.

The ProFaba activities help strengthen and align European faba bean research, as exemplified by the genome assembly effort and the use of a common genotyping platform and data repository. ProFaba project results will rapidly be taken up by the breeder partners, accelerating the development of new and improved faba bean cultivars for all European regions.

#### **Keywords**

Genetics · genotype by environment interaction · legume · protein crop · *Vicia faba*

#### **Acknowledgements**

The project ProFaba was carried out under the ERA-NET Cofund SusCrop (Grant N°771134), being part of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE -JPI).

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## Calorespirometry as a phenotyping tool for selection of genotypes with high plasticity for temperature stress: analysis of pea seeds

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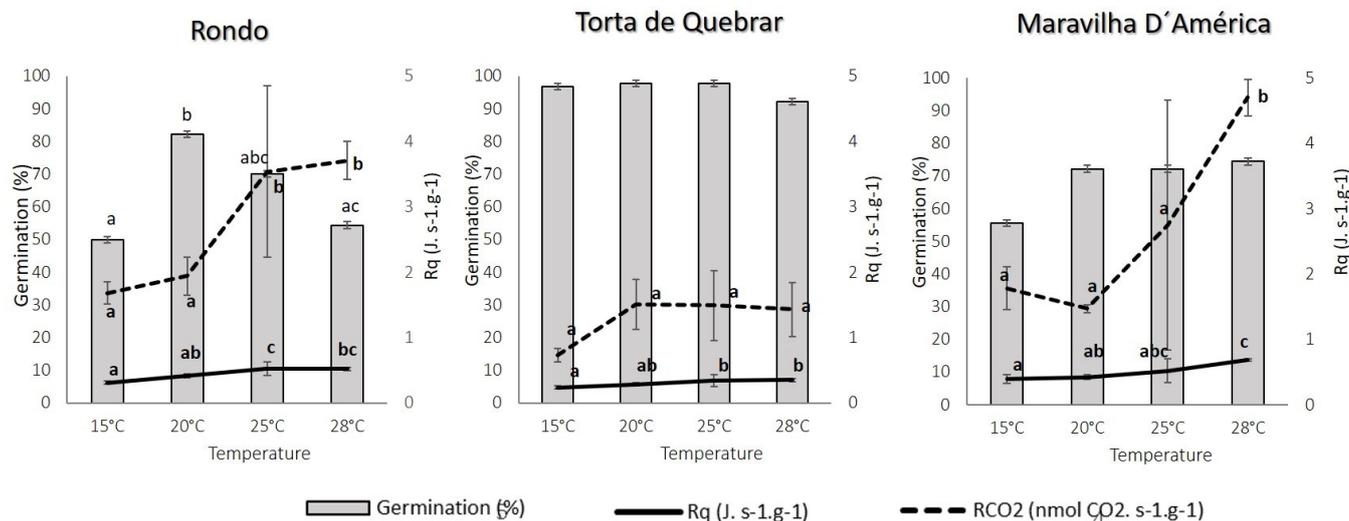
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Increasing human population and climatic and edaphic changes impose needs to develop new cultivars with seeds that have greater plasticity for environmental constraints and higher germination rates. Improving seed vigor remains a primary objective of the agricultural industry and the seed/breeding companies that support it. Phenotyping tools to assist breeding programs for phenotyping seeds for resilience and selecting high-quality crop seeds are of interest. Because temperature is the major environmental constraint controlling growth, with a strong impact on crop yield, we have investigated the temperature dependence of respiratory parameters with calorespirometry as an alternative to conventional seed phenotyping strategies. Calorespirometry has been used to assess metabolic and respiratory changes associated with cell reprogramming events, and has been proposed as a screening tool for predicting growth phenotype for temperature stress. Calorespirometry measures metabolic heat rates ( $R_q$ ) and CO<sub>2</sub> emission rates ( $R_{CO_2}$ ) of biological samples as a function of temperature allowing prediction of growth rates ( $R_{struct\ biomass}$ ) without growing plants under different environmental temperatures. Calorespirometry also allows the determination of the fraction of carbon substrate incorporated into structural biomass, *i.e.* carbon use efficiency ( $\epsilon$ ), as a function of temperature.

Three pea (*Pisum sativum* L.) cultivars ('Rondo', 'Torta de Quebrar' and 'Maravilha D'América') commonly used in conventional agriculture were selected for calorespirometry measurements and germination trials. The effect of temperature on seed germination was evaluated after an imbibition period of 16 h under dark conditions. Calorespirometric measurements were conducted in isothermal mode in a Multi-Cell Differential Scanning Calorimeter (TA Instruments, New Castle, DE) at 15, 20, 25 and 28°C. A minimum of three measurements were made on each cultivar. The heat rates,  $R_q$ , were measured directly and CO<sub>2</sub> rates,  $R_{CO_2}$ , were deter-

mined from the increase in the heat rate in the presence of a vial of NaOH, which reacts with CO<sub>2</sub> in an exothermic reaction (-108.5 kJ/mol CO<sub>2</sub>). For data validation, a seed germination trial with the same cultivars was conducted under the same temperatures tested in the calorimeter. Substrate carbon conversion efficiencies and growth rates could not be calculated from the data collected in this study because the calorespirometric ratios,  $R_q/R_{CO_2}$ , showed that a significant portion of the CO<sub>2</sub> was produced by anaerobic respiration.

At 20, 25 and 28°C,  $R_q$  was significantly lower in cv. 'Torta de Quebrar' than in the other two cultivars. At 15°C, no significant differences were detected among the three cultivars. All three cultivars have significantly higher  $R_q$  values at 25 and 28°C than at 15 and 20°C (Figure 1).  $R_{CO_2}$  exhibited lower values for cv. 'Torta de Quebrar' than the other two cultivars.  $R_{CO_2}$  in cv. 'Torta de Quebrar' was constant from 15 to 28°C. In contrast, cvs. 'Rondo' and 'Maravilha D'América' exhibited an increase in  $R_{CO_2}$  with increasing temperature. In the germination trial, no significant differences were observed in the percentage of germination at the four temperatures for cvs. 'Torta de Quebrar' and 'Maravilha D'América'. In contrast, cv. 'Rondo' exhibited the highest germination rate at 20 and 25°C, and the lowest germination rate at the extreme temperatures, 15 and 28°C (Figure 1). The cv. 'Torta de Quebrar' presented the highest germination rates, above 90% across the temperature range, while in cv. 'Maravilha D'América' germination was around 70%. In cv. 'Rondo', the maximum germination rate was achieved at 20-25°C (70-80%) (Figure 1). This indicates that cv. 'Torta de Quebrar' has the highest germination rate and the highest resilience in germination across temperatures. In agronomical terms, this result means a high and stable production, which highlights cv. 'Torta de Quebrar' as the most resilient against temperature stress.



**Figure 1** Results of  $R_q$ ,  $R_{CO_2}$  and germination rates achieved under different temperatures for the three *Pisum sativum* cultivars

In the calorimetric measurements, cv. 'Torta de Quebrar' showed the lowest values of both  $R_q$  and  $R_{CO_2}$  and in the germination trial, the highest germination rates, suggesting that early metabolic homeostasis for carbon and energy metabolism further modulates plant cell development and growth. The first hours of seed germination are characterized by the stress generated by water uptake. During the imbibition period, mitochondria, peroxisomes and the plasma membrane NADPH oxidases represent the main sources of reactive oxygen species (ROS) generation. To avoid damage to the embryo that could negatively impact germination, ROS levels must be strictly controlled by antioxidant systems. The reduction of metabolism and efficient mechanisms for ROS scavenging may represent an efficient strategy to overcome the stress imposed on cells. Validation of this hypothesis requires further experiments to evaluate the efficiency of antioxidant mechanisms in seeds of cv. 'Torta de Quebrar' in comparison with the other two cultivars. We propose that low  $R_q$  values in seeds during the early stages of germination are correlated with high resilience for temperature stress. The present work demonstrates the applicability of calorimetry to assess seed vigor at different temperatures, suggesting this method as a reliable phenotyping tool for selecting more resilient genotypes.

#### Keywords

Germination · *Pisum sativum* · resilience · respiration · stress

#### Acknowledgements

This work was supported by the EU project LIVESEED - Improve performance of organic agriculture by boosting organic seed and plant breeding efforts across Europe funded by the European Union's HORIZON 2020 research and innovation programme under the Grant Agreement no 727230, and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090; and by National Funds through FCT under the

Project UIDB/05183/2020. Authors are very thankful to IIFA for the fellowship given to L. Rodrigues. The opinions expressed and arguments employed herein do not necessarily reflect the official views of the EC and the Swiss government. Neither the European Commission/SERI nor any person acting behalf of the Commission/SERI is responsible for the use which might be made of the information provided in this publication.

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## Estimation of soybean seed protein accumulation by measuring canopy hyperspectral reflectance

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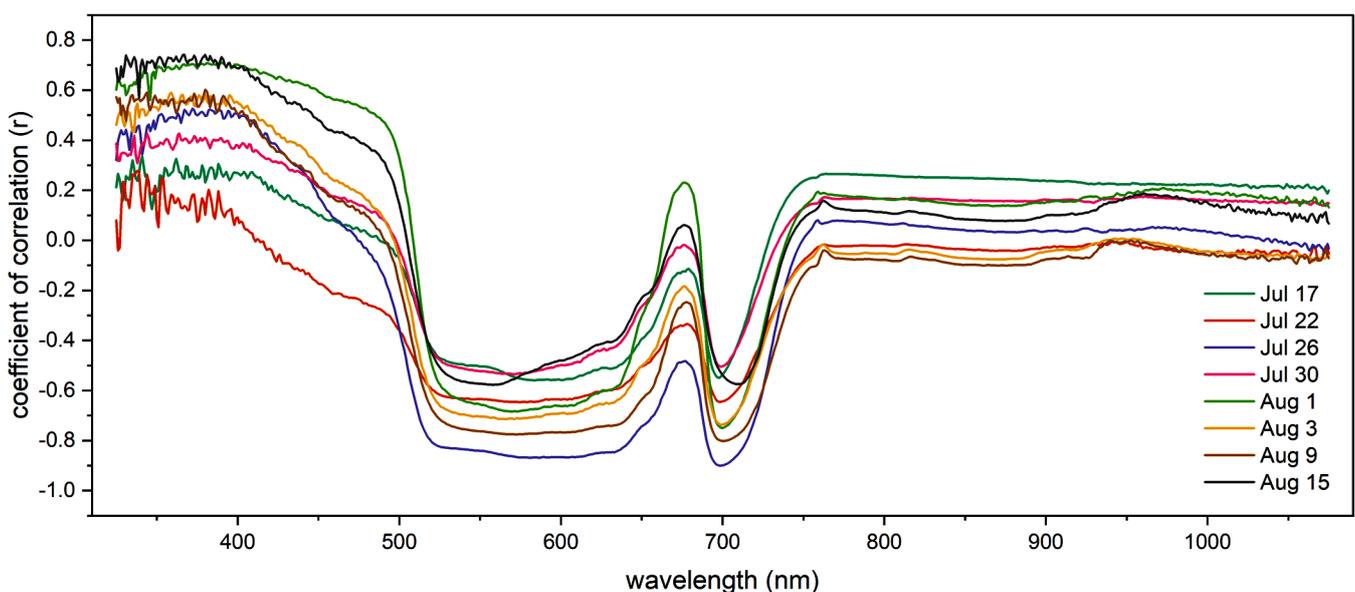
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Soybean (*Glycine max* (L.) Merr.) is the major source of plant-based protein for both human nutrition and animal feeding. In Central European countries, interest in organic soybean production has recently grown due to increasing demands for high protein raw materials in food production. Consequently, dinitrogen fixation by symbiotic rhizobial bacteria is playing a crucial role for yield performance and harvest product quality. Additionally, high levels of symbiotic nitrogen fixation are particularly relevant for achieving a positive nitrogen balance as well as a beneficial crop rotation effect of soybean in organic production systems.

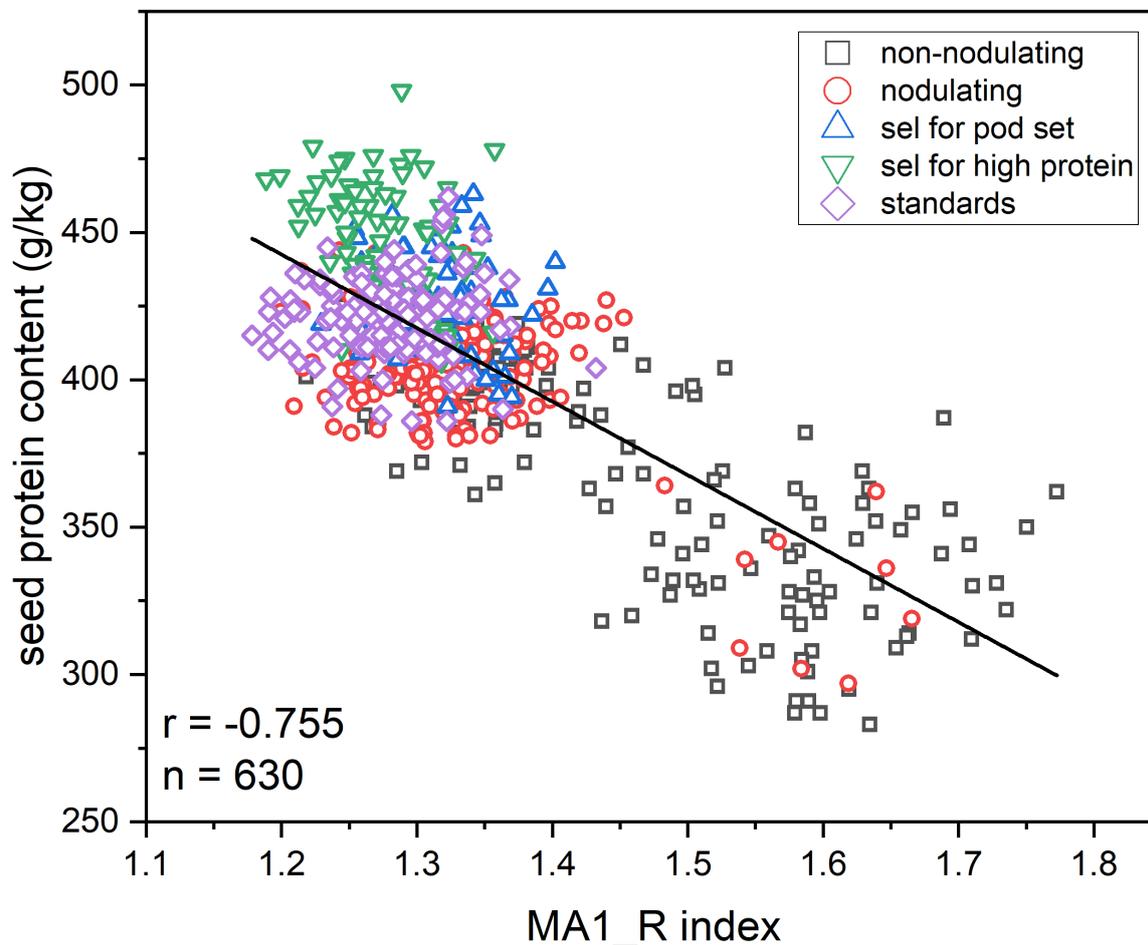
As direct methods of measuring nitrogen fixation under field conditions are tedious and not suitable for high-throughput screening of genotypes within plant breeding programs, the present research aimed at evaluating indirect phenotyping methods for field-based screening of soybean nitrogen uptake with seed protein content as a target trait. A set of soybean genotypes widely differ-

ing in seed protein content was tested in three environments (Tulln 2019, Tulln 2020, Raasdorf 2020) in the east of Austria in replicated single-row plots. Between the soybean developmental stages of full flowering (R2) and full seed (R6), *i.e.* the seed filling period, canopy hyperspectral reflectance data were collected at about weekly time intervals using a hand-held spectroradiometer (ASD HandHeld 2 Fieldspec,  $\lambda$  325-1075 nm). Reflectance data at particular wavelength points were then utilized for calculating indices or regression models for predicting seed protein content.

In individual experiments, a level of seed protein content in the wide range of 285-489 g/kg was found. Reflectance data at particular wavelength regions were clearly correlated to seed protein content (Figure 1). From over 40 spectral reflectance indices calculated, nitrogen reflectance index (NRI), greenness index (GI) and different ratio vegetation indices (RVI) with optimized wavelengths for soybean canopies revealed highest correlations



**Figure 1** Relationship between hyperspectral reflection during the soybean seed filling period and seed protein content of the harvest product: Correlograms describing correlations between reflectance at given wavelengths (1 nm increment) and seed protein content for the Tulln 2019 subset A (nodulating vs. non-nodulating population) at 8 different spectral data collection dates.



**Figure 2** Relationship between hyperspectral reflection during the soybean seed filling period and seed protein content of the harvest product: Regression of the MA1\_R index term on seed protein content for all genotypes and across all environments.

( $r = 0.88$ ) to seed protein content within particular environments. These indices were also highly correlated with leaf chlorophyll content (SPAD-meter-values). The ratio vegetation index term MA1\_R showed the highest correlation to seed protein content across all three environments and all genotypes (Figure 2). In a second approach utilizing the whole range of spectral data available and partial-least-square regression modelling (PLSR), correlations of up to  $r = 0.91$  (model calibration;  $r = 0.89$  for model validation) were achieved for seed protein content data across all three environments. In addition to seed protein content which is directly related to nitrogen fixation, hyperspectral reflectance data appeared to be useful for prediction of additional traits such as time to maturity, oil content or 1000-seed weight as well. Moreover, on the level of individual genotypes, particular indices could be utilized for better characterization of genotypes in terms of water use efficiency, biomass production, and nitrogen metabolism.

While further modelling and application research will be required for optimizing the screening procedures, the present results reveal a considerable potential of hyperspectral reflectance measurement for characterizing soybean traits related to nitrogen content. This could contribute to selection for improved nitrogen fixation capacity and better harvest product quality in organic soybean production.

#### Keywords

Field phenotyping · *Glycine max* · hyperspectral reflectance · nitrogen fixation · seed protein content · soybean breeding

#### Acknowledgements

The research leading to these results has partly received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement number 771367. The support from BOKU project "Phenotyping across experimental scales" (IA 13460) is also acknowledged.

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## ADAPT - Accelerated development of multiple-stress tolerant potato

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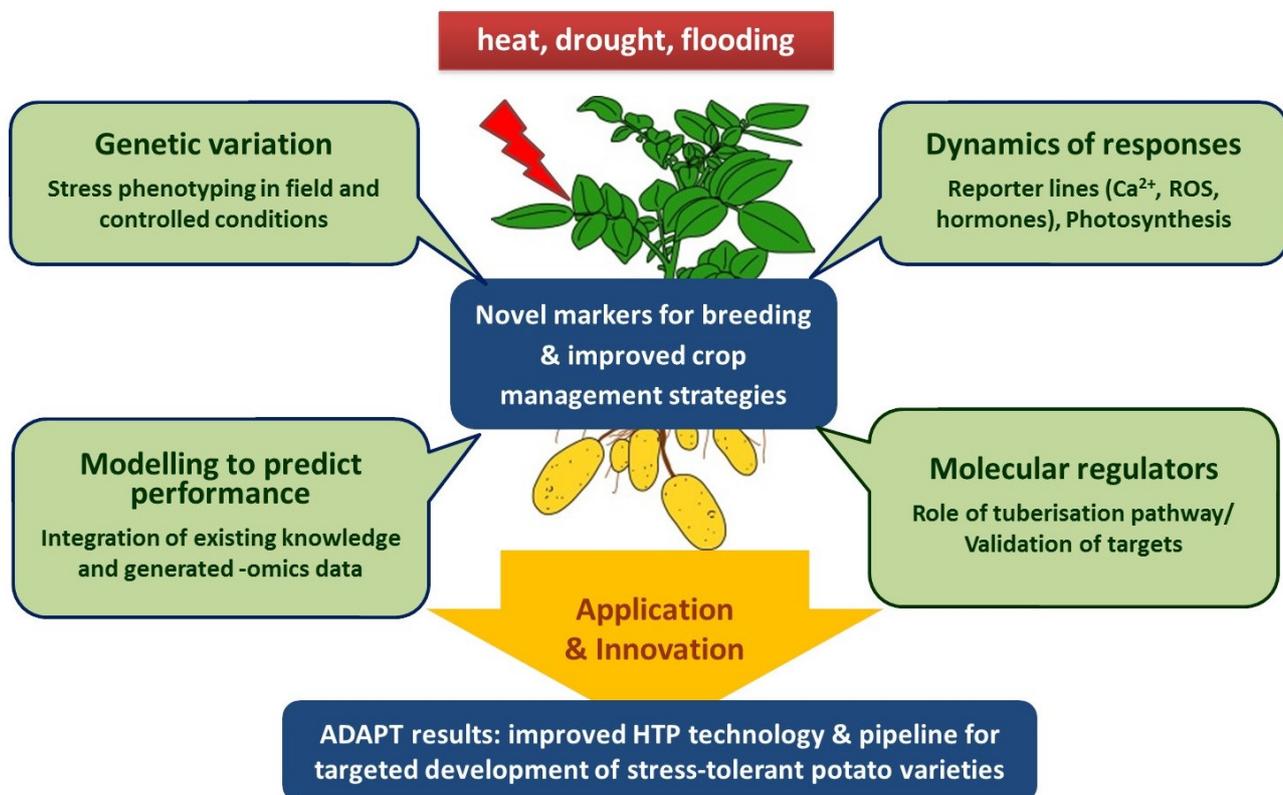
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Potato is one of the most important food crops worldwide. Abiotic stresses associated with climate change, like heat, drought and flooding, severely impact yields. The project ADAPT (Accelerated Development of multiple-stress tolerAnt PoTato) aims to support the potato breeding community by gathering new knowledge about the crop's molecular and phenotypic adaptation processes towards environmental stresses. ADAPT combines the academic

and applied expertise of 17 project partners. Seven universities and three research institutions study plant abiotic stress physiology, signalling networks and molecular modelling. Three potato breeders are actively involved, and a screening technology developer facilitates direct implementation of the results in practice. The European Potato Trade Association (Europatat; europatat.eu) and the Austrian Agency for Health and Food Safety (AGES;



**Figure 1** ADAPT uses various experimental approaches to facilitate marker development and breeding of stress-tolerant potato varieties, including recommendations for improved crop management. (HTP, high throughput phenotyping; Ca<sup>2+</sup>, calcium; ROS, reactive oxygen species)

www.ages.at) support the dissemination and exploitation of research results by reaching out to the end-users.

The project aims to determine the molecular and phenotypical responses of potatoes to combined environmental stress conditions, including the identification of molecular mechanisms for better adaptation to combinations of heat, drought and flooding stress in potato. Accordingly, eight distinct work packages combine methods in molecular biology, stress physiology, systems biology and analytics with molecular breeding and sophisticated engineering. The integration of the gained knowledge into modern breeding programmes and agricultural management practises for yield stability under combinatorial stress conditions is the central aim of ADAPT. For further information and details, please visit the ADAPT website: <https://adapt.univie.ac.at/>

Workpackage 6 ("Pathways to impact") focusses on the validation, the exploitation and the implementation of the research results, and comprises a plan for communication and dissemination. As a basis for further breeding and marker development, the potential target genes that were identified in previous work packages will be validated. In addition, interesting and easily assessable phenotypic traits will be selected based on the results of the other work packages and further validated in local field trials. The aim is to develop recommendations for phenotypic traits to be included in standard value of cultivation and use (VCU) protocols to determine abiotic stress tolerance in potato varieties. When implemented, these assessments will add to variety descriptions and sup-

port end-users in their variety choice. To engage with breeders and farmers, European potato farmers are invited to participate in an online survey. This survey focusses on the perception of changing climatic conditions and the needs of potato farmers to cope with the challenging growing conditions due to climate change. With this knowledge, recommendations for potato breeding strategies in the context of climatic stresses will be developed. In addition, field days will be organized in cooperation with the breeding companies involved in the project. The goal is to connect farmers, breeders and researchers, discuss future needs and show the implementation of new technologies in breeding activities. By improving variety assessment and tools like the AGES Variety Finder with the incorporation of abiotic stress tolerance in the variety descriptions, farmers and breeders will be able to find suitable varieties in line with their individual growing conditions and requirements.

#### **Keywords**

Breeding · climate change · *Solanum tuberosum* · stress tolerance · variety testing

#### **Acknowledgements**

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. GA 2020 862-858.



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